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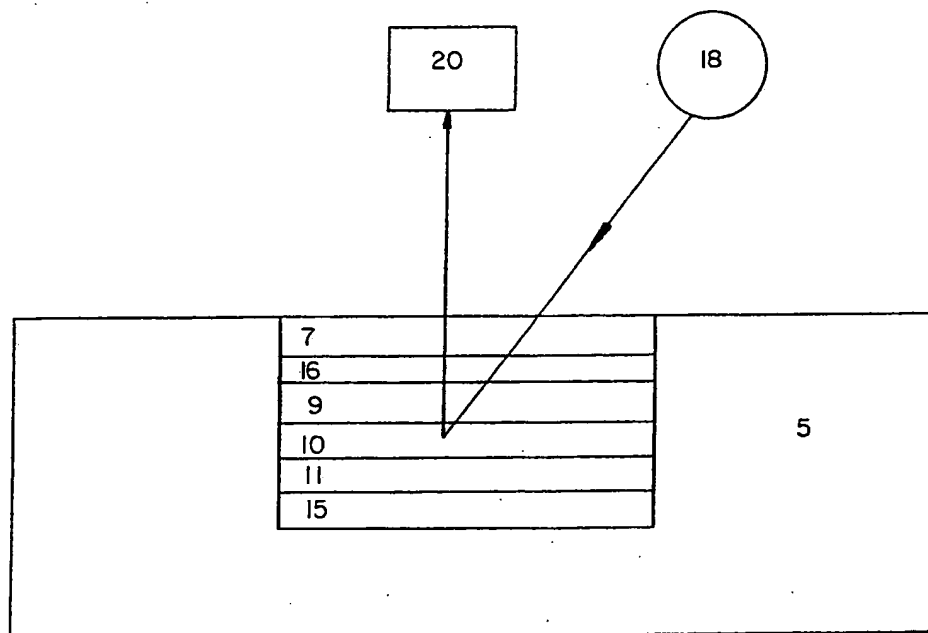
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(54) Title: HYDROGEL DYE FILM SENSING ELEMENTS AND THEIR PREPARATION



(57) Abstract

Reactive azo dyes, hydrogels, dye films (9) prepared therefrom, and sensing elements incorporating such films are disclosed. The sensing elements are useful, e.g., in body fluid analyzing devices, where they are capable of providing accurate information on the pH or pCO<sub>2</sub> of fluids such as blood.

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HYDROGEL DYE FILM SENSING ELEMENTS  
AND THEIR PREPARATION

FIELD OF THE INVENTION

5           The present invention relates to new hydrogels, azo dyes, and pH sensitive dye films and sensing elements made therefrom. More particularly, it relates to hydrogel dye film sensing elements capable of detecting pH and pCO<sub>2</sub>, and to apparatus useful for in  
10   vivo measurement of the pH and pCO<sub>2</sub> of body fluids, such as blood.

BACKGROUND OF THE INVENTION

          Although body fluid analyzers are known in the art, presently available analyzers use electro-  
15   chemical sensors, also known as electrodes, to measure blood parameters such as pH and pCO<sub>2</sub>. See, e.g., U.S. Patents 3,874,850; 4,097,921; 4,579,641; 4,615,340. Such electrode measuring techniques often require a great deal of costly equipment and frequent  
20   maintenance. Typically, an elaborate 2-point calibration is required. Acceptable accuracy with reusable electrochemical systems is obtained only with an additional step of the cleaning electrodes with a washing solution after each use.

25           Optical sensors provide an alternative to electrochemical sensors, and there is a consequent need for new materials useful for making reliable optical

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sensing elements which are compatible with available optical equipment.

#### SUMMARY OF THE INVENTION

It is an object of this invention to provide  
5 a sensing element for body fluid analysis that is reliable, accurate and easy to use.

It is another object to provide a sensing element for optical pH and pCO<sub>2</sub> measurement which has an effective rejection of incident light.

10 It is another object to provide a sensing element which provides an accurate measurement of pH and pCO<sub>2</sub>, that is independent of both indicator concentration and optical transfer function.

It is a further object of this invention to  
15 provide new component materials that are suitable for making sensing elements for optical pH or pCO<sub>2</sub> of fluids.

It is another object to provide a pH indicator dye which responds to all pH changes by  
20 changing to a color which can be detected optically.

It is another object to provide an indicator dye which absorbs light of wavelengths greater than 500 nm.

It is another object to provide a reactive  
25 dye which can be incorporated by a variety of linking groups into synthetic polymers, which polymer-bound dyes, in turn, can function as useful pH-indicators.

It is another object to provide novel hydrogels which are compatible with optical dye  
30 systems.

It is another object to provide hydrogels into which dyes may be permanently incorporated and which are suitable for use in optical sensing elements.

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It is further object to provide hydrogel dye films capable of undergoing an optically detectable, reversible color change as a function of changing pH.

These and other objects are accomplished  
5 herein by providing a dye-containing sensing element, e.g. for use in an apparatus for analyzing body fluids, the apparatus providing for contact between the sensing element and the fluid to be analyzed, and providing optical elements for automatically reading color  
10 changes in the sensing elements. The sensing elements according to the present invention are comprised of thin films of a polyurethane or polyacrylamide hydrogel containing one or more azo dye indicators. The azo dye indicators undergo reversible color shifts as a  
15 function of pH, and apparatus equipped with the sensing elements of the invention can optically detect such color shifts and calibrate the shifts to give accurate information as to the pH and pCO<sub>2</sub> of the fluid contacting the sensing elements.

20 The polyurethane or polyacrylamide hydrogels and azo dyes disclosed herein are employed to form the sensing elements of the present invention.

#### BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 is a schematic diagram of a fluid  
25 analyzer apparatus according to the invention.

FIGURE 2 is a sectional plan view of a possible configuration for a sensing cuvette in accordance with the invention, employing two sensing cells having sensing elements therein for detecting,  
30 respectively, pH and pCO<sub>2</sub> of a fluid.

FIGURE 3 is a sectional plan view of a sensing cell employing a sensing element according to the present invention showing its relationship toward

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impinging light from the optical components of a fluid analyzer which measures backscattered light.

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to apparatus  
5 for analyzing fluids and to dyes, hydrogels, and dye films useful in the sensing elements in such apparatus.

Preferred sensing elements of the present invention are useful for detecting pH or for detecting  $\text{pCO}_2$ . Each of the sensing elements comprises one or  
10 more thin films. The films are most advantageously deposited in layers on an optically clear substrate and disposed within fluid analyzer sensing cells such that the outermost layer is contacted by the fluid to be analyzed. For detecting changes in pH and  $\text{pCO}_2$ , the  
15 sensing elements depend on a dye film layer, which is normally the first layer of the sensing element (i.e., deposited directly on the optically clear substrate). The sensing elements may also include light scattering film layers and light absorbing film layers, to permit  
20 the use of optical equipment that measures backscattered light, as opposed to transmitted light. In  $\text{pCO}_2$ -detecting sensing elements, a gas permeable layer will also be employed.

The arrangement of the layers will depend on  
25 the configuration of the optical reading elements of the apparatus. For example, in the preferred embodiment described in detail herein, in which light backscattered through the dye film is measured by a photodetector, the order of the layers will be as  
30 follows: optically clear substrate, dye film layer, light reflective layer, light absorptive layer, gas permeable layer ( $\text{pCO}_2$ -detecting elements only). As will be seen more clearly in the drawings, the optically clear substrate is oriented toward the light source,

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and the outermost film layer (e.g., the gas permeable layer or the light absorptive layer) communicates with the channel of the sensing cuvette through which the fluid to be measured is made to pass.

### 5 The Azo Dyes

The indicator dyes used to produce the pH-detecting dye films of the present invention are azo dyes of the general formula:



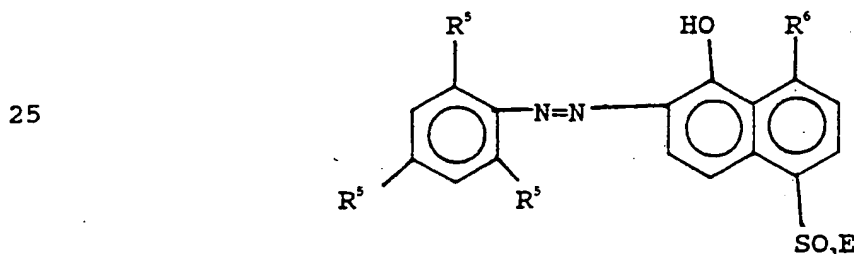
10 wherein:

$R^2$  represents a phenyl, naphthyl, or a  $C_2$ - $C_{12}$  heterocyclic aromatic radical, which may be substituted with one or more groups selected from nitro, cyano, sulfo, carboxy, carboxamido, carboalkoxy, acyl, alkoxy, 15 perfluoroalkyl, or halogen atoms such as bromine, chlorine, fluorine, etc.;

$R^3$  represents a sulphonated naphthol or sulphonated aminonaphthol radical; and

20  $R^4$  represents a reactive substituent capable of binding the dye molecule to a polymeric substrate without affecting the pH-indicating character of the dye.

Preferred dyes have the formula (I):



wherein:

Each  $R^5$  is independently selected from 30 hydrogen, halogen (chlorine, bromine, fluorine, etc.), perfluoroalkyl of 1 to 4 carbon atoms, nitro, sulfo,

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cyano, carboxy, carboalkoxy of 1 to 4 carbon atoms, carboxamido, or acyl, provided that at least one R<sup>5</sup> is nitro or cyano;

R<sup>6</sup> is amino, carboxamido, acrylamido,  
5 -NHCO(CH<sub>2</sub>)(CH<sub>2</sub>OH)<sub>2</sub>, or -NHCOCH(NR'<sup>6</sup>)CH<sub>2</sub>NR'<sup>6</sup>, wherein R'<sup>6</sup> and R<sup>6</sup> are, independently, hydrogen, straight chain or branched alkyl, aminoalkyl, or hydroxyalkyl groups of 1 to 6 carbon atoms (e.g., methyl, ethyl, propyl, t-butyl, isobutyl, isopentyl, aminoethyl, aminopropyl,  
10 hydroxyethyl, hydroxypropyl, tris(hydroxymethyl)methyl, and the like), or R' and R<sup>6</sup> taken together form the radical -CH<sub>2</sub>CH<sub>2</sub>NR'<sup>6</sup>CH<sub>2</sub>CH<sub>2</sub>-, wherein R'<sup>6</sup> is hydrogen or any of the aforementioned C-C<sub>6</sub> aminoalkyl or hydroxy-alkyl radicals; and

15 E is hydrogen, sodium, lithium, potassium, magnesium or calcium.

Although preferred functional groups, R<sup>6</sup>, are explicitly defined above, also contemplated are other groups which will serve to bind the dye molecules to a  
20 substrate without interfering with its pH-indicating properties. In addition to amido linking groups mentioned above, hydrocarbons (e.g., alkylene), ether and thioether bridges terminating in a variety of functional groups may also be employed.

25 Most preferred are dyes in which R<sup>5</sup> para to the azo group and on R<sup>5</sup> ortho to the azo group are or cyano nitro, and R<sup>6</sup> is -NHCO(CH<sub>2</sub>)(CH<sub>2</sub>OH)<sub>2</sub>, -NHCOCH<sub>2</sub>NR'<sup>6</sup>, or -NHCOCH(NR'<sup>6</sup>)CH<sub>2</sub>NR'<sup>6</sup>, and R' and R<sup>6</sup> are aminoethyl, aminopropyl, hydroxyethyl, hydroxypropyl, or tris  
30 (hydroxymethyl)methyl.

The reactive azo dyes of the present invention exhibit a pH-dependent color change with absorption maxima at wavelengths longer than 500 nm for both the protonated and deprotonated forms of the dye,  
35 and there is nearly complete spectral separation of the



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protonated and the deprotonated forms. A single sharp transition due to a protonated equilibrium is exhibited within the pH-range encountered with physiological fluids such as blood; and the dyes are stable under the  
5 variable conditions of the medical environment in which they can be advantageously used (i.e., in terms of stability to heat, light, oxygen, compatability with physiological fluids, stability to sterilization procedures).

10 The dyes are conveniently incorporated into the structure of the polyurethane hydrogels and thereby immobilized, preventing them from leaching or bleeding out of the hydrogel substrate during use.

The dyes having the structure of formula (I)  
15 have a red to blue transition with increasing pH.

The dyes may be advantageously prepared via a coupling reaction between diazonium salts and amides derived from aminonaphthol sulfonic acids. The preferred starting materials for the dyes of formula  
20 (I) thus include the 2-halo-4,6-dinitro-phenyldiazonium salts and their analogues (see R<sup>5</sup> in formula (I)). Azo dyes resulting from the coupling reaction of 2-fluoro-4,6-dinitrophenyldiazonium sulfate, 2-chloro-4,6-dinitrophenyldiazonium sulfate, 2-bromo-4,6-  
25 dinitrophenyldiazonium sulfate, or 2-trifluoromethyl-4,6-dinitrophenyldiazonium sulfate and amides derived from the following aminonaphthol sulfonic acids have pK<sub>a</sub> values between 6 and 8 and undergo yellow/orange/red to purple/blue color changes with increasing pH: 4-amino-  
30 5-hydroxy-1-naphthalenesulfonic acid, 6-amino-4-hydroxy-2-naphthalenesulfonic acid, 6-amino-7-hydroxy-2-naphthalenesulfonic acid, 7-amino-4-hydroxy-2-naphthalene sulfonic acid.

The pK<sub>a</sub> can be lowered and the water-  
35 solubility increased by the introduction of a second

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sulfonic acid group on the naphthalene ring. Thus, amides derived from 3-amino-5-hydroxy-2,7-naphthalenedisulfonic acid or 4-amino-5-hydroxy-2,7-naphthalenedisulfonic acid (H-Acid) may be used as  
5 coupling components.

In the absence of sulfonic acid substituents or equivalent groups, the solubility of the dye in water is so greatly reduced that hydrophobic interaction of the indicator with the supporting  
10 substrate is expected to be prominent.

Generally, with respect to the selection of substituted diazonium components, a variety of substituents may be selected, depending on the properties desired in the final dye. For example,  
15 nitro, cyano, sulfo, carbonyl, carboxamido, carboalkoxy, acyl, methyl- or methoxy-substituted phenyl or naphthyl diazonium reagents may be used. Hydroxy substitution is also possible, however those dyes containing an ortho hydroxy substituent form complexes with heavy  
20 metal ions, a characteristic which may render them useless as pH-indicators. Although hydroxy substituents in the meta or para positions have less of a chelating effect, for the purposes described herein, hydroxy substituents on the diazonium coupling  
25 component are to be avoided. The position and electronegativity of the substituents can be used to adjust the  $pK_a$  value of the final dye. However, the actual  $pK_a$  value may also be further modified by the nature of the reactive group (e.g.,  $R'$ ,  $R''$ , supra) and  
30 by subsequent binding of the azo dye to a substrate.

In formula (I) type dyes, substituents on the phenyl ring are preferred to be in conjugation with the hydroxyl group because of a bathochromic effect ("red shift"). Nitro groups in those positions also lower  
35 the  $pK_a$  substantially.

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The chemical nature of the reactive group R' (R' in formula (I)) of the azo dye must be sufficiently reactive to allow incorporation of the dye in the structure of the urethane hydrogel, and it must not be electronically coupled with the pi-system. For the preferred dyes, amidation of the naphthol derivatives can be effected by methods which are known (see, for example, J. Benz et al., U.S. Patent 3,114,746, and G. Back et al., U.S. Patents 4,502,865, 4,432,898 and 3,625,935), and can be carried out prior or subsequent to diazonium coupling. For example, the amide derivatives may be prepared by dropwise addition of the acylating agent to an aqueous solution of the azo dye or the starting amino-naphthol (di)sulfonic acid. The pH of the solution can be maintained by addition of a solution of sodium hydroxide or by the presence of sodium hydrogen carbonate resulting in a weakly acidic to weakly alkaline medium. The reaction stoichiometry should be carefully controlled to avoid acylation of the hydroxyl group.

Diazonium coupling is carried out using standard methods (see, for example, H. E. Fierz-David and L. Blangey, "Fundamental Processes of Dye Chemistry", translated by P. W. Vittum, Interscience Publishers, Inc: New York, 1949; p. 239 ff). In most cases diazotization is advantageously carried out using nitrosyl sulfuric acid in concentrated sulfuric acid (see G. Roebisch et al., J. Prakt. Chem. 320, 1047-54 (1978); cf. U.S. Patent 3,931,142; Japan Kokai JP 83-152056 and JP 83-160357; Chemical Abstracts 100: 69852e and 87240h). Coupling is generally rapid at 0°C in acidic aqueous solutions or slurries of the coupling components. If necessary, the product dyes are precipitated by addition of salt, and further purification is possible by washing with water followed

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by washing with organic solvent such as acetone and methanol; recrystallization from methanol may be used to further improve purity.

Amidation prior to diazonium coupling is preferred if coupling is desired ortho or para to the hydroxyl substituent (as in formula (I) above). However, useful pH-indicator dyes are also obtained by coupling ortho or para to the amino substituent, in which case an amino group is left that can be used for subsequent binding of the dye to a substrate. For example, such dyes are readily incorporated into isocyanate-capped polyurethane prepolymers, however this normally results in a radical shift in the optical characteristics of the dye (concomitant with engaging the free amino group) which makes this option somewhat less desirable for end uses such as optical pH or pCO<sub>2</sub> measurement. For the same reason electrophilic reactive substituents, such as aromatic sulfonyl halides or isocyanates, are less desirable. Ideally, there is a spacer group between the reactive group and the indicator dye for electronic uncoupling as well as for minimally restricted molecular motion of the bound dye.

Examples of suitable acylating reagents that result in reactive groups, R', of electrophilic character are acryloyl chloride, 2,3-dibromopropanoyl chloride, 2,3-dichloropropanoyl chloride, bromoacetyl chloride, etc. Reactive dyes obtained using these reagents can be bound directly to a substrate that contains nucleophilic substituents, or they can be readily converted into stable derivatives by treatment with suitable nucleophiles such as methylamine, ethanolamine, N-alkylethanolamines, diethanolamine, tris(hydroxymethyl) aminomethane, ethylenediamine, piperazine, and the like. The addition of substituents

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bearing hydroxyl or amino functions is most preferred, as it permits ready binding of the dye into the structure of polyurethane substrates by reaction with electrophilic groups such as isocyanates.

- 5           The 2,3-dihalopropanoamide derivatives are readily converted to 2-haloacrylamides at pH 6 or above. Dyes bearing acrylamido or 2-haloacrylamido groups may be linked covalently to a matrix by copolymerization with monomers such as acrylamide.
- 10 This can be accomplished by heat curing which results in some thermal degradation.

          Alternatively, R' (R') may be a substituted amido group such as 2,2-bis(hydroxymethyl)propanoamido. In yet another embodiment, R' is an amide derived from a

15 succinamido substituent, for example an amide formed between an alkanolamine or diamine and the free carboxyl group of the succinamide.

- It is also possible to electrostatically bind the dyes via their charged substituents. In this case
- 20 no reactive group R' is required unless it is used to introduce additional charged groups for increased affinity to a charged substrate. For example, a charged group may be introduced by reaction of the acylated dye with aspartic acid. The electrostatic
- 25 binding of the dye may strongly affect the pK-value and can be used to modify the pH-sensitive range for color change after binding to a substrate. Examples are the binding of sulfonated dyes to a quarternarized poly(ethyleneimine)-containing polymer. However,
- 30 binding indicator dyes electrostatically via salt bridges is somewhat less desirable than covalent bonding because of the high probability of the formation of different micro-environments which affect the spectral characteristics of the dye.

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The following examples illustrate the preparation of azo dyes according to the invention. The examples are not to be construed as limiting the invention.

5

EXAMPLE 1

4-(2-bromoacrylamido)-5-hydroxynaphthalenesulfonic acid, sodium salt (ANS-2-bromoacrylamide):

Amide formation was carried out in water, with simultaneous addition of the acid chloride and 1N NaOH (or solid NaOH pellets) to maintain the pH between 6 and 7. Thus, 4-amino-5-hydroxy-1-naphthalenesulfonic acid (S-Acid, 47.85 g, 200 mmol) was dissolved in 500 ml water containing NaOH (10 g, 250 mmol), and the pH adjusted to 6.5 with concentrated HCl. The solution was stirred vigorously and 2,3-dibromopropanoyl chloride (200 mmol, 50.06 g, 23.3 ml) was added dropwise over 30 minutes; solid NaOH is added simultaneously to maintain the pH at  $6.5 \pm 1$ , and NaOH addition was continued until the pH stabilized (up to one additional hour, about 20 g of NaOH total was required). At this point, conversion of the 2,3-dibromopropanoamide to the 2-bromo-acrylamide was complete. The pH was reduced to 4 using concentrated HCl. Cooling in ice gave a dense precipitate which was collected on filter paper and washed with 20% aqueous salt solution (about 100 ml) to remove dark colored impurities. The product was purified by extraction into hot ethanol (500 ml), leaving behind residual salt and unreacted starting material; most of the ethanol was then evaporated to precipitate the product as a yellow powder, which was washed with ethanol and acetone. The yield after drying under vacuum at 80°C is 17.82 g (45 mmol, 23%).

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EXAMPLE 2Diazotization of 2-bromo-4,6-dinitroaniline:

A solution of the diazonium salt was obtained by dissolving 2-bromo-4,6-dinitroaniline (6 mmol, 1.57 g) in concentrated sulfuric acid (11 g, 6 ml) followed by addition of nitrosylsulfuric acid (0.76 g, 6 mmol). The yellow-orange solution was stirred for 1 hour at room temperature.

EXAMPLE 3B.-2-bromoacrylamide:

ANS-2-bromoacrylamide (2.38 g, 5 mmol) was dissolved in 100 ml water. The solution was cooled to 0°C by the addition of crushed ice. Approximately 0.5 equivalents of diazonium solution (2.5 ml, 2.5 mmol) was added to the stirred solution. After stirring overnight, the solution was acidified to pH 3. The addition of a sufficient 20% aqueous salt solution gave a 10% salt solution and precipitated the product.

The purple-red precipitate was washed with a small amount of water, dried and then taken up in hot methanol. Most of the solvent evaporated. The addition of ether gave a microcrystalline product (0.80 g, 1.1 mmol) (43% based on the diazonium salt, 21% based on the starting amide) which was free of most impurities by liquid chromatographic analysis (silica, eluting with BuOH, EtOH, NH<sub>4</sub>OH--3:2:1).

In the following example, 4-amino-5-hydroxy-1-naphthalenesulfonic acid is converted to the ANS-2-bromoacrylamide and then coupled with the same diazonium reagent used in the previous example, without recrystallization of the intermediate ANS-2-bromoacrylamide.

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EXAMPLE 4B.-2-bromoacrylamide:

4-amino-5-hydroxy-1-naphthalenesulfonic acid (210 g, 875 mmol) was dissolved in 2.8 L water containing NaOH (40 g, 1 mol) and the pH adjusted to 6.5 with concentrated HCl; it was then treated with the acid chloride (105 ml, about 895 mmol), with the simultaneous addition of NaOH pellets to control the pH. The product started to precipitate before the end of acid chloride addition. Once the pH stabilized at 5, NaCl (300 g) was added to complete precipitation of the product, which was washed with 20% aqueous NaCl. It was then taken up in 3 L water containing KOH (pH 10) and heated to 70°C. This solution was filtered to remove insoluble black material. The resulting solution was acidified in 1 l batches with concentrated HCl (30 ml, to pH 1.5), cooled by addition of 1 kg crushed ice, and treated with 225 ml of the diazonium solution while maintaining vigorous stirring. The temperature rose to near room temperature during mixing, and significant nitrogen evolution was observed. The product was collected on filter paper (a very slow process), then slurried twice with water (about 500 ml each time), followed by filtration (very slow). The product was further washed with methanol (2x 500 ml) acetone (250 ml), and finally ether (500 ml). The yield after vacuum drying was 139.6 g (209 mmol, 24%).

EXAMPLE 5A.-2-bromoacrylamide:

Diazotization of 2,4-dinitroaniline was carried out on a 25 mmol scale (4.58 g) in concentrated sulfuric acid (20 g) containing nitrosylsulfuric acid



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(3.18 g, 25 mmol) at room temperature. The solution was stirred for one hour and then diluted with ice (60 g). It was then poured into a slurry of the ANS-2-bromoacrylamide (25 mmol, 9.85 g) in a mixture of ice (60 g) and concentrated sulfuric acid (20 g). A deep red color appeared immediately. After one hour the product was collected by filtration, slurried with water, and refiltered.

Thin Layer Chromatography (TLC) analysis (silica, eluting with BuOH:EtOH:NH<sub>4</sub>OH--3:2:1) revealed two major products: a faster-eluting, yellow component and a more polar red component. The red product was obtained in pure form by recrystallization from methanol. After drying, the yield was 3.69 g (6.3 mmol, 25%). This dye exhibited a red/blue transition with a pK<sub>a</sub> of 8.8 (in 50% methanol-aqueous buffer mixtures). The yellow component was partially purified by evaporation of the methanol and extraction of the residue into tetrahydrofuran, followed by precipitation with ether (yield 1/74 g, 3.0 mmol, 12%). It exhibited a yellow/blue transition at pH 8.5.

#### EXAMPLE 6

##### A,-2-bromoacrylamide:

2-chloro-4,6-dinitroaniline (2.18 g, 10 mmol) was diazotized in concentrated sulfuric acid (10 g) containing nitrosyl-sulfuric acid (1.27 g, 10 mmol) at room temperature. After one hour this solution was diluted with ice (30 g) and poured into a well-stirred slurry of the ANS-2-bromoacrylamide (3.94 g, 10 mmol) in a mixture of ice (50 g), water (25 g) and concentrated sulfuric acid (25 g). Coupling proceeded rapidly to give a deep red product which was recovered by filtration after 1.5 hours. After washing with water, methanol, acetone, and ether, this solid was

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recrystallized from a mixture of methanol/2-butanone. The microcrystalline product was washed with acetone and ether (2.59 g, 4.3 mmol, 43%). The dye displayed a sharp red/blue transition at pH 6.7.

5

EXAMPLES 7-10

(A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub>, A<sub>4</sub>)-2-bromoacrylamides:

Diazonium coupling with 2,6-dinitro-4-trifluoromethylaniline (A<sub>1</sub>-2-bromoacrylamide); 2,6-dinitroaniline (A<sub>2</sub>-2-bromoacrylamide); 2,4-dinitro-6-trifluoromethylaniline (A<sub>3</sub>-2-bromoacrylamide) and 4-carboxamido-2,6-dinitroaniline (A<sub>4</sub>-2-bromoacrylamide). The methods of Examples 4-6 were used to couple these diazonium components with the ANS-2-bromoacrylamide. All of the resulting dyes were useful pH indicating compounds, capable of further reaction at the 2-bromoacrylamido group, permitting their incorporation into polymeric films. The properties of these dyes are summarized in Table 1, *infra*.

Additional dyes were prepared from the ANS-2-bromoacrylamide which incorporate heterocyclic diazonium components, or diazonium components bearing a variety of other functional groups. These dyes were prepared by the methods described in the previous examples, and their properties are summarized in Table 2, *infra*.

EXAMPLE 11

4-(2-bromoacrylamido)-5-hydroxy-2,7-naphthalenedisulfonic acid, disodium salt (ANDS-2-bromoacrylamide):

2,3-dibromopropanoyl chloride (40 mmol, 4.6 ml) was added to a well-stirred solution of 4-amino-5-hydroxy-2,7-naphthalenedisulfonic acid (H-Acid, 40 mmol of the monosodium salt sesquihydrate, 14.73 g) in 150

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- ml water adjusted to pH 6.5 with 1N NaOH over 15 minutes at room temperature. Simultaneously, 1N NaOH was added to maintain the pH between 6 and 7. When the reaction was completed (indicated by a stable pH),
- 5 solid NaOH was added to raise the pH to 12, and the solution was heated briefly to boiling. The mixture was then cooled and acidified to pH 3 with concentrated HCl. Addition of NaCl (50 g) produced a precipitate which was extracted with boiling ethanol (2x250 ml).
- 10 Upon cooling, the extract deposited a precipitate which was mostly NaCl. The filtered extract was concentrated and gave the desired product as a light-yellow solid (2.85 g). The off-white residue from the extraction (15.62 g after drying) also contained the desired
- 15 product, along with 5.5 equivalents of NaCl according to elemental analysis. The yield was 24.8 mmol (62%).

#### EXAMPLE 12

##### Diazonium coupling with 2,4-dinitroaniline:

- Diazotization was carried out by adding 2,4-
- 20 dinitroaniline (0.92 g, 5 mmol) to a stirred solution of nitrosylsulfuric acid (0.64 g, 5 mmol) in concentrated H<sub>2</sub>SO<sub>4</sub> (10 g) at room temperature. After one hour this solution was diluted with ice (30 g) and poured into a well-stirred slurry of the ANDS-2-
- 25 bromoacrylamide (5 mmol, 2.48 g) in water (20 ml), ice (40 g) and concentrated H<sub>2</sub>SO<sub>4</sub> (20 g). A cherry-red product formed immediately. After one hour it was recovered by centrifugation. Extraction into acetone afforded about 0.2 g of a dark-red solid which turned
- 30 purple above pH 10. The insoluble residue (0.1 g) from this extraction exhibited an orange/blue transition at pH 6.

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EXAMPLE 13Diazonium coupling with 4-nitro-1-aminonaphthalene:

Diazotization was carried out on 2.5 mmol (0.47 g) of 4-nitro-1-aminonaphthalene in ethanol (40 ml) containing ice (40 g), concentrated HCl (10 ml) and NaNO<sub>2</sub> (0.173 mg, 2.5 mmol). After two hours at 0°C this solution was added to the ANDS-2-bromoacrylamide (1.24 g, 2.5 mmol) dissolved in 10 ml water plus 10 ml ethanol, which had been acidified with concentrated HCl (2 ml) and cooled to 0°C. Two products gradually formed (according to TLC analysis on silica, eluting with BuOH:EtOH:NH<sub>4</sub>OH--3:2:1) a very polar purple-blue component and a less polar purple component (the latter exhibited a transition to blue at pH 11).

15

EXAMPLE 14Diazonium coupling with 2-chloro-4,6-dinitroaniline:

2-chloro-4,6-dinitroaniline (5 mmol, 1.09 g) in small portions was added to a stirred solution of nitrosylsulfuric acid (5 mmol, 0.64 g) in concentrated sulfuric acid (10 g) at room temperature. After one hour this solution was diluted with ice (30 g) and then poured into a vigorously stirred slurry of the ANDS-2-bromoacrylamide (5 mmol, 2.48 g in 20 ml water, 40 g ice and 20 g concentrated H<sub>2</sub>SO<sub>4</sub>). The reaction mixture immediately turned red, and a cherry-red precipitate was collected after 1.5 hours. This product was washed with 20% aqueous NaCl and then extracted with hot methanol (2x100 ml). After concentration, this extract yielded an orange-red solid (0.50 g) which exhibited an orange-blue transition in solution at pH 6. Further concentration yielded a deep-red solid (0.48 g) which exhibited a red-purple transition at pH 8.5. The undissolved residue from this extraction (0.91 g), also

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exhibited this red-purple transition. The combined yield of the two products was 2.8 mmol (56%).

#### EXAMPLE 15

##### Diazonium coupling with 2-bromo-4,6-dinitro-aniline:

5 Using conditions similar to those described for the corresponding chloro-substituted aniline, two dyes were obtained with nearly identical characteristics as pH indicators.

#### EXAMPLE 16

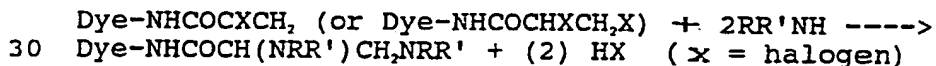
##### 10 5-nitroanthranilonitrile:

This compound was diazotized on a 5 mmol scale (0.82 g) as in Examples 14 and 15, and coupled with the ANDS-2-bromoacrylamide (5 mmol, 2.48 g) as previously prepared in Example 11. The rapid reaction  
15 gave two major products in a combined yield of 1.17 g (1.9 mmol, 37%). The less polar, orange component turned blue above pH 6, while the more polar, red component exhibited a transition to purple with a pK<sub>a</sub> of roughly 5.

20 Table 3, infra, summarizes the properties of the dyes prepared according to Examples 12-16.

Table 4, infra, summarizes examples of pH-indicating dyes prepared from a variety of other coupling components.

25 The addition of organic amines to dyes bearing either the 2,3-dihaloacrylamido or 2-haloacrylamide groups is presumed to occur according to the following equation:



The R and R' substituents on the amine are chosen to provide covalent or electrostatic binding of the dye to the hydrogel (covalent linkage is

- 20 -

preferred); their choice also influences the solubility of the dye, a significant consideration in the preparation of dye-containing polymers.

EXAMPLE 175 B.-bis(diethanolamine):

When the B<sub>5</sub>-2-bromo-acrylamide (0.5 mmol, 0.33 g) was treated with a five-fold excess of diethanolamine (2.5 mmol, 0.26 g) in 15 ml methanol at room temperature, a slow reaction ensued. TLC analysis  
10 (silica, eluting with BuOH:EtOH:NH<sub>4</sub>OH--3:2:1) showed that the adduct degrades under the reaction conditions, and it was not isolated.

EXAMPLE 18B.-bis(ethanolamine) :

15 B<sub>5</sub>-2-bromoacrylamide (15 mmol, 10.01 g) was treated with five equivalents of ethanolamine (75 mmol, 4.59 g) in methanol (250 ml) at room temperature for 1 hour. After filtration to remove insoluble impurities, the reaction mixture was concentrated to a volume of  
20 50 ml. Acidification with glacial acetic acid gave a purple precipitate which was washed with methanol, acetone and finally ether. The yield after drying was 5.53 g.

A second yield (0.50 g) was obtained by  
25 further reducing the solution volume (8.8 mmol, 59%). The dye had a pK<sub>a</sub> of about 6.7, with absorption maxima at 515 nm (pH 4) and 605 nm (pH 10). An isosbestic point occurred at roughly 535 nm.

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EXAMPLE 19B<sub>2</sub>-bis(methylamine):

The B<sub>2</sub>-2-bromoacrylamide (2 mmol, 1.33 g) in methanol (100 ml) was treated with aqueous methylamine (8 mmol, 0.62 g of a 40% solution, diluted with 80 ml methanol) overnight at room temperature. TLC analysis (silica, eluting with BuOH:EtOH:NH<sub>4</sub>OH--3:2:1) indicated complete conversion of the starting material. The reaction mixture was acidified with acetic acid, filtered, and reduced in volume to 50 ml. The product precipitated upon addition of acetone (100 ml). The yield was 0.47 g (0.72 mmol, 36%). The dye had a pK<sub>a</sub> very close to 7.

EXAMPLE 2015 B<sub>2</sub>-bis(N-methylethanolamine):

N-methylethanolamine (75 mmol, 5.63 g) was added to the B<sub>2</sub>-2-bromo-acrylamide (15 mmol, 10.01 g) in methanol (250 ml) at room temperature. The resulting blue solution was stirred for 1 hour. TLC analysis (silica, eluting with BuOH:EtOH:NH<sub>4</sub>OH--3:2:1) showed the reaction to be complete, with a single product spot at R<sub>f</sub> 0.40 replacing the starting material at R<sub>f</sub> 0.45. The solution was filtered through Whatman No. 1 paper, and then reduced in volume to about 35 ml on the rotary evaporator. Addition of acetone (250 ml) gave a purple precipitate (6.73 g, 63%) which was washed with acetone. Its purity was at least 80% by High Pressure Liquid Chromatography (HPLC), with a less polar impurity representing the balance of the material. Its pK<sub>a</sub> in aqueous buffers was approximately 8. Absorption maxima occurred at 625 nm (pH 10) and 530 nm (pH 4), with an isobestic point at 555 nm.

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EXAMPLE 21B<sub>2</sub>-bis(N-methylethanolamine):

This dye can also be precipitated in two acidified forms. After reaction with N-methylethanol-  
amine as described in Example 20, treatment of the  
reaction mixture with glacial acetic acid gave a high  
yield (73%) of a red powder, presumably protonated at  
the naphthol end but not at the sulfonate end. This  
form of the dye was poorly soluble in water or organic  
solvents.

EXAMPLE 22B<sub>2</sub>-bis(Tris):

B<sub>2</sub>-2-bromoacrylamide (25 mmol, 16.68 g) was  
treated with five equivalents of tris(hydroxy-  
methyl)aminomethane (125 mmol, 15.14 g) in water  
(750 ml) at 45°C overnight. Conversion was monitored  
by TLC (silica gel, eluting with BuOH:EtOH:NH<sub>4</sub>OH--  
3:2:1). The product was recovered by acidification of  
the reaction mixture with acetic acid, followed by  
filtration and washing with water. Recrystallization  
from methanol gave a purple product which was washed  
with acetone and ether (9.67 g, 48%). Its pK<sub>a</sub> in aqueous  
buffer was approximately 6.9, with absorption maxima at  
515 nm (pH 4) and 605 nm (pH 10), and an isobestic  
point at 530 nm.

EXAMPLE 23B<sub>2</sub>-bis(Tris):

B<sub>2</sub>-2-bromoacrylamide (5 mmol, 3.34 g) was  
treated with five equivalents of tris(hydroxy-  
methyl)aminomethane (25 mmol, 3.03 g) in 50 ml methanol  
at 50°C overnight. Conversion was monitored by TLC  
(silica gel, eluting with BuOH:EtOH:NH<sub>4</sub>OH--3.2.1). The



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product was recovered by acidification of the reaction mixture with acetic acid, followed by filtration and washing with a methanol-acetone mixture and then ether to give a purple powder (1.63 g, 40%).

5

EXAMPLE 24Addition of diamines to B,-2-bromoacrylamide:

Diamino compounds including ethylene diamine, piperazine, and N-2-hydroxyethylpiperazine were added to the reactive dye in aqueous or methanol solution at  
10 room temperature, using a four to five-fold excess of the diamine. These reactions were completed within 15 minutes, and the adducts were isolated in high yield (>80%). However, TLC analysis (silica, eluting with BuOH:EtOH:NH<sub>4</sub>OH--3:2:1) revealed a mixture of products.  
15 Furthermore, these adducts had significantly lower pK<sub>a</sub>'s (<7), making them less suitable as physiologic pH indicators.

EXAMPLE 25A,-bis(methylamine):

20 A,-2-bromoacrylamide (2 mmol, 1.18 g) was treated with methylamine (8 mmol, 0.62 g of a 40% aqueous solution) in 50 ml methanol and stirred at room temperature overnight. The resulting red precipitate was collected on a glass frit, washed with methanol and  
25 acetone, and dried under vacuum at 60°C. The yield was 0.94 g (1.7 mmol, 86%). This dye exhibited a clean red/blue transition at pH 9.5.

EXAMPLE 26A,-bis(Tris):

30 Tris(hydroxy-methyl)aminomethane (10 mmol, 1.21 g) was reacted with A,-2-bromoacrylamide (2 mmol,

- 24 -

1.18 g) in methanol (25 ml) which required about 24 hours at 60°C. The product, a red powder, was recovered by filtration and washed with methanol, acetone, and ether, followed by drying under vacuum at 5 80°C. The yield was 1.19 g (1.6 mmol, 80%). This dye's spectral characteristics included maxima at 605 nm (pH 11) and 510 nm (pH 7), with an isobestic point at 535 nm. Its  $pK_a$  was 9.2.

#### EXAMPLE 27

##### 10 A<sub>2</sub>-bis(Tris):

A<sub>2</sub>-2-bromoacrylamide (2 mmol, 1.25 g) reacted with tris(hydroxy-methyl)aminomethane (10 mmol, 1.21 g) in methanol (25 ml) at 60°C overnight. TLC analysis (silica, eluting with BuOH:EtOH:NH<sub>4</sub>OH--3:2:1) indicated 15 that same starting material still remained, so a small amount of Tris (121 mg) was added and the reaction mixture stirred at 60°C for two more hours. The product was recrystallized from a methanol-acetone solution to give 0.32 g (0.4 mmol, 20%) of impure 20 material. Its  $pK_a$  was 6.7, and absorption maxima were observed at 515 nm (pH 4) and 615 nm (pH 10), with an isobestic point at 540 nm.

The following example describes the introduction of the bromoacetamide reactive group into 25 the coupling component.

#### EXAMPLE 28

##### ANS-2-bromoacetamide:

A slurry of 4-amino-5-hydroxy-1-naphthalenesulfonic acid (S-acid, 50 g, 209 mmol) in 30 540 ml water was adjusted to pH 6.5 using a 30% solution of sodium hydroxide. The solution was cooled to 10°C using an ice bath. Bromoacetyl chloride

- 25 -

(32.9 g, 209 mmol) was added dropwise over half an hour with good stirring. The pH was maintained at 6.5 by addition of sodium hydroxide solution. The pH stabilized 20 minutes after the acid chloride addition was complete, and a heavy precipitate formed. The reaction mixture was acidified to pH 3 using concentrated hydrochloric acid, and the precipitate was collected by filtration. The solid product was reslurried in salt water (20% aqueous NaCl, 500 ml), and again collected on a filter. The solids were then slurried in acetone (500 ml), collected by filtration, and dried at 60°C under vacuum for four hours. The yield is 42 g (52%). Further purification is accomplished through recrystallization from hot ethanol.

The following examples illustrate the preparation of dyes from the coupling component bearing the 2-bromoacetamide group.

EXAMPLE 29

20 B.-2-bromoacetamide:

The diazonium reagent was prepared as in example 2, from 2-bromo-4,6-dinitroaniline (20 mmol, 5.24 g) and nitrosylsulfuric acid (20 mmol, 2.54 g) in concentrated sulfuric acid (40 g). Before use, the diazonium solution was diluted with 120 g crushed ice. The resulting yellow solution was added in one portion to the coupling component (ANS-2-bromoacetamide, 20 mmol, 7.64 g) in a well stirred slurry containing water (40 ml), crushed ice (80 g) and concentrated sulfuric acid (40 g). After stirring for one half hour, the red-purple solid was collected on filter paper. It was reslurried in 200 ml of brine (20% aqueous NaCl), collected by filtration, and washed with

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salt water (50 ml) and then water (50 ml). After air drying, the solid product is further purified by slurrying in warm methanol (250 ml), filtration, and washing with an additional 200 ml of methanol. The  
5 yield after vacuum drying is 7.27 g (55%).

#### EXAMPLE 30

##### A,-2-bromoacetamide:

A diazonium solution was prepared by adding 2-chloro-4,6-dinitroaniline (2.17 g, 10 mmol) to  
10 concentrated sulfuric acid (20 g) containing nitrosylsulfuric acid (10 mmol, 1.27 g). After stirring for one hour, the dark yellow diazonium solution was diluted with crushed ice (60 g) and poured into a well-stirred slurry containing ANS-2-  
15 bromoacetamide (10 mmol, 3.82 g), water (20 ml), crushed ice (40 g), and concentrated sulfuric acid (20 g). After one hour, the solid product was collected by filtration, reslurried in 10% aqueous NaCl (50 ml), again filtered, and washed with water (50 ml).  
20 The dye was further purified by dissolving it in methanol (1 L) containing 10 mmol sodium hydroxide (10 ml of a 1 N aqueous solution), followed by filtration and acidification with concentrated hydrochloric acid. The solution was concentrated to  
25 about 75 ml, and the resulting precipitate was collected on a fritted glass funnel, dispersed in water (25 ml), refiltered, washed with water and methanol, and dried under vacuum at 70°C. The yield was 4.29 g (70%).

30

#### EXAMPLE 31

##### A,-2-bromoacetamide:

A diazonium solution was prepared by adding 2-trifluoromethyl-4,6-dinitroaniline (5.02 g, 20 mmol)

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to concentrated sulfuric acid (40 g) containing nitrosylsulfuric acid (20 mmol, 2.54 g). After stirring for one hour, the dark yellow diazonium solution was diluted with crushed ice (120 g) and  
5 poured into a well-stirred slurry containing ANS-2-bromoacetamide (20 mmol, 7.64 g), water (40 ml), crushed ice (80 g), and concentrated sulfuric acid (40 g). After one hour, the solid product was collected by filtration, reslurried in water (150 ml)  
10 and precipitated by the addition of 20% aqueous NaCl (150 ml), again filtered, and washed with water (50 ml). It was further purified by recrystallization from hot methanol (1 L) to give, after vacuum drying, 1.57 g (12%) of a golden-red solid.

15

EXAMPLE 32A-2-bromoacetamide:

A diazonium solution was prepared by adding 2-amino-3,5-dinitrobenzenesulfonic acid (5.70 g, 20 mmol as the sodium salt) to concentrated sulfuric  
20 acid (40 g) containing nitrosylsulfuric acid (20 mmol, 2.54 g). After stirring for one hour, the dark yellow diazonium solution was diluted with crushed ice (120 g) and poured into a well-stirred slurry containing ANS-2-bromoacetamide (20 mmol, 7.64 g), water (40 ml),  
25 crushed ice (80 g), and concentrated sulfuric acid (40 g). After stirring overnight, the solid product was collected by filtration, reslurried in water (125 ml) and precipitated by the addition of 20% aqueous NaCl (125 ml), again filtered, and washed with  
30 water (50 ml). The product was further purified by recrystallization from hot methanol (500 ml), the metallic gold-green crystals being collected by filtration after reduction of the volume of the methanol solution to about 50 ml at the rotory

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evaporator. After drying, the yield was 6.47 g (9.5 mmol, 48%).

To facilitate their incorporation in polyurethane hydrogels, the 2-bromoacetamine derivatives were reacted with diethanolamine (DEA) to give 2-(DEA)-acetamide derivatives. The two hydroxyethyl substituents are capable of reaction with isocyanates to form urethane linkages.

#### EXAMPLE 33

##### 10 B<sub>2</sub>-2-(DEA)-acetamide:

B<sub>2</sub>-2-bromoacetamide (5 mmol, 3.28 g) was treated with diethanolamine (25 mmol, 2.63 g) in tetrahydrofuran (125 ml). The reaction was nearly complete after stirring for one hour at room temperature, according to TLC analysis (silica, eluting with BuOH:EtOH:NH<sub>4</sub>OH--3:2:1). The resulting blue precipitate was collected on a glass fritted filter, and further purified by stirring with methanol (50) followed by filtration, washing with methanol (10 ml) and drying under vacuum at 65°C. The yield was 2.82 g (4.15 mmol, 83% assuming the dye is a sodium salt).

#### EXAMPLE 34

##### A<sub>2</sub>-2-(DEA)-acetamide:

A<sub>2</sub>-2-bromoacetamide (2 mmol, 1.22 g) was treated with diethanolamine (10 mmol, 1.05 g) in methanol (50 ml). The reaction was incomplete after stirring for one day at room temperature, according to TLC analysis (silica, eluting with BuOH:EtOH:NH<sub>4</sub>OH--3:2:1). After four days, a purple precipitate was collected on a glass fritted filter. The filtrate was evaporated to a small volume and the resulting blue precipitate collected by filtration, and dried under

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vacuum at 65°C. The yield was 0.89 g (1.35 mmol, 68% assuming the dye is a sodium salt).

Acidifying the filtrate with glacial acetic acid led to the recovery of an additional 0.17 g of  
5 solid (0.27 mmol as the zwitterion, 13%).

#### EXAMPLE 35

##### A<sub>2</sub>-2-(DEA)-acetamide:

A<sub>2</sub>-2-bromoacetamide (2 mmol, 1.29 g) was treated with diethanolamine (10 mmol, 1.05 g) in  
10 tetrahydrofuran (50 ml) with stirring at room temperature. The reaction was nearly complete after 15 minutes, according to TLC analysis (silica, eluting with BuOH:EtOH:NH<sub>4</sub>OH--3:2:1). After 45 minutes, methanol (50 ml) was added to dissolve the sticky mass  
15 of product. The resulting solution was filtered through a medium glass fritted funnel, leaving a small amount of insoluble residue which was washed with additional methanol. The filtrate was acidified with concentrated hydrochloric acid and the resulting red  
20 precipitate collected by filtration, washed with methanol, and vacuum dried. The yield was 0.76 g (1.2 mmol, 59%).

#### EXAMPLE 36

##### A<sub>2</sub>-2-(DEA)-acetamide:

25 A<sub>2</sub>-2-bromoacetamide (5 mmol, 3.39 g) was treated with diethanolamine (25 mmol, 2.63 g) in tetrahydrofuran (125 ml). Reaction was slow on account of the insolubility of the dye in tetrahydrofuran, so methanol (50 ml) was added. The reaction was nearly  
30 complete after stirring for two days at room temperature, according to TLC analysis (silica, eluting with BuOH:EtOH:NH<sub>4</sub>OH--3:2:1). A green-gold precipitate was collected (0.50 g), which proved to be residual

- 30 -

starting material (15%). The filtrate was evaporated and the residue taken up in water; the product was precipitated by addition of brine (100 ml of aqueous NaCl). The precipitate was collected on a glass  
5 fritted filter and washed with water (20 ml). It was further purified by recrystallization from methanol (1.5 L) by removal of most of the solvent at the rotary evaporator. After drying under vacuum at 65°C, the yield was 1.35 g (2.0 mmol, 40%).

10

EXAMPLE 37B.-succinamide:

The diazonium solution (from  
2-bromo-4,6-dinitroaniline: 20 mmol, 5.24 g) was prepared in the usual manner (Example 29), and coupled  
15 with ANS-succinamide (20 mmol, 7.23 g) in a well-stirred mixture of water (40 ml), crushed ice (80 g), and concentrated sulfuric acid (40 g). The resulting red precipitate was collected on Whatman #4 paper and washed with water. It was reslurried in water (100 ml)  
20 to which was then added 20% aqueous NaCl (100 ml), and the resulting precipitate collected by filtration and washed with water. The product was recrystallized from methanol to give green-gold microcrystals (5.58 g, 44%) after vacuum drying.

25

Table 5 (*infra*) summarizes the properties of the dyes prepared according to Examples 29-37.

EXAMPLE 37ANS-succinamide:

A solution of succinic anhydride (250 mmol,  
30 25.02 g) in acetone (200 ml) was added over 30 minutes to a solution of 4-amino-5-hydroxy-1-naphthalene-sulfonic acid (S-acid, 59.80 g, 250 mmol) in 500 ml water containing sufficient sodium hydroxide (about



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10 g) to adjust the pH to 6.5; the pH was maintained at 6.5  $\pm$  0.2 by addition of 50% aqueous sodium hydroxide solution. The reaction was rapid, and the pH stabilied within a few minutes after anhydride addition was  
5 complete. The product was recovered by adding salt (100 g) and then acidifying the solution to pH 3 with concentrated hydrochloric acid; the resulting precipitate was collected by filtration on Whatman #5 paper. The solid product was washed with 20% aqueous  
10 NaCl and then a small amount of water. It was then taken up in 1.25 L of hot ethanol, filtered, and the volume reduced to give off-white crystals (total yield 56.02 g, 62%).

The following example describes the  
15 incorporation of the bis(2-hydroxymethyl)propanoamido substituent into the coupling component.

#### EXAMPLE 39

##### ANS-BHPA:

4-(1-methyl-4-oxo-3,5-dioxo-4-thiacyclohexane)  
20 carbonyl chloride (24.77 g, 125 mmol, prepared according to U.S. Patent 3,770,770) was added dropwise over 30 minutes to a well stirred solution of 4-amino-5-hydroxy-1-napthalenesulfonic acid (S-acid, 29.9 g, 125 mmol) in 250 ml water containing 5.0 g NaOH at room  
25 temperature. The initial pH was adjusted to 6.5 with concentrated hydrochloric acid. The pH was maintained at 6.5  $\pm$  0.2 by the addition of 50% aqueous NaOH solution. The reaction was judged complete when the pH reading stabilized about 15 minutes after addition of  
30 the acid chloride was complete.

The reaction mixture was then acidified to pH 0.5 with concentrated HCl and heated at 80°C for 2 hours. High performance liquid chromatography (HPLC)

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analysis was used to determine when cleavage of the cyclic sulfite was complete. The solution was then filtered while warm using Whatman #54 filter paper and Celite. An HPLC sample was run to determine ANS-BHPA concentration.

Examples 40-44 disclose the synthesis of dyes bearing the bis(2-hydroxymethyl)propanoamido group.

#### EXAMPLE 40

##### Diazonium Solution Preparation:

The diazonium solution was prepared by adding 2-bromo-4,6-dinitroaniline (32.75 g, 125 mmol, Aldrich) to a solution of nitrosylsulfuric acid (15.88 g, 125 mmol, Aldrich) in 50 ml concentrated sulfuric acid at room temperature. The resulting solution was stirred at room temperature for one hour. The diazonium solution can be stored at -20°C for several days.

#### EXAMPLE 41

##### B.-BHPA:

One equivalent of the diazonium solution was cautiously diluted 3:1 with water at -10°C and then slowly added to a vigorously stirred solution of ANS-BHPA (170 ml, 50 mmol) containing 100 g ice. The reaction was allowed to warm to room temperature and filtered using Whatman #54 filter paper. This filtration took several hours. The resulting material was slurried in 200 ml of water to which was added 100 ml of 20% aqueous NaCl. The slurry was filtered (Whatman #54 filter paper) and then washed with water (2 L total). The cake was allowed to air dry for approximately 3 hours. The material was then slurried with 250 ml acetone and filtered (Whatman #54 filter paper). Finally, the dye was slurried with 100 mL

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methanol and filtered (Whatman #54 filter paper) and then vacuum dried at 60°C for 24 hours. The yield was 12.0 g (37%), and the its purity by HPLC was 96%.

EXAMPLE 425 A<sub>1</sub>-BHPA:

A diazonium solution was prepared by addition of 2-chloro-4,6-dinitroaniline (25 mmol, 5.44 g) to concentrated sulfuric acid (40 g) containing nitrosylsulfuric acid (25 mmol, 3.18 g). It was  
10 diluted with crushed ice (120 g) and added in one portion to a solution of ANS-BHPA (25 mmol in 60 ml water) to which crushed ice (120 g) had been added. The dense red-purple precipitate was collected by filtration overnight on Whatman #54 paper. It was then  
15 dispersed in water (300 ml), precipitated with 20% aqueous NaCl (300 ml), and filtered on Whatman #54 paper. The resulting cake was washed with water (100 ml) and air dried. It was further purified by dispersion in acetone (225 ml), followed by filtration  
20 and then recrystallization from methanol. After vacuum drying, the yield was 6.68 g (44%) and HPLC analysis indicated a purity of 92%.

EXAMPLE 43A<sub>1</sub>-BHPA:

25 The required aniline (4,6-dinitro-2-fluoroaniline) was prepared as described by Schiemann and Miou (Ber. 66, 1179-87 (1933)). It was diazotized on the same scale used in the previous example (25 mmol, 5.03 g), the only difference being that the  
30 diazonium solution could not be diluted with ice before use. Rather, it was added slowly to the well-stirred ANS-BHPA solution (60 ml, 25 mmol) containing crushed ice (180 g). Work-up and purification were identical

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to that described in the preceding example. The yield was 3.81 g (26%) after vacuum drying; purity was 92% according to HPLC.

#### EXAMPLE 44

##### 5 A<sub>17</sub>-BHPA:

The aniline (2-bromo-6-cyano-4-nitroaniline) was prepared according to U.S. Patent 3,821,276 (Mrozik and Bochis). Its diazotization on a 40 mmol scale (9.68 g) was carried out in concentrated sulfuric acid (40 g) containing one equivalent of nitrosylsulfuric acid (40 mmol, 5.08 g) at room temperature. Just before use, the diazonium solution was diluted with crushed ice (120 g), and then added to a well-stirred solution of ANS-BHPA (39 mmol in 170 ml water) containing additional crushed ice (100 g). The reaction mixture turned brown. Filtration on Whatman #541 paper, followed by washing with water (150 ml total), afforded a deep red product, which was further purified by slurrying with acetone (300 ml), filtration, and then recrystallization from methanol.

The properties of these and other BHPA dyes similarly prepared are listed in Table 6 (infra)

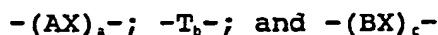
#### The Hydrogels

The film layers utilized in the present invention, and, in particular, the pH-sensing dye films, are comprised of polyurethane or polyacrylamide hydrogels. By "hydrogel" is meant a water-swollen (or swellable) three-dimensional matrix or network of crosslinked, hydrophilic macromolecules. Fully swollen hydrogels will contain 20% to over 90% water.

The polyurethane hydrogels of the present invention are a copolymerization product of alkylene glycols or thioglycols, organic polyisocyanates, and

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optionally, ionic diols, such as sulfonate diols, quaternary ammonium diols, carboxylate diols, and phosphate diols, or acids corresponding to such diols. Preferred hydrogels according to the present invention have a three-dimensional network structure and include polymeric units of the following general formulas:



wherein:

AX represents a urethane derived by the reaction of a polyalkylene glycol or thioglycol (A) of molecular weight 200 to 15,000 (preferably 600 to 3000) with an organic diisocyanate or triisocyanate (X); T represents a trifunctional or tetrafunctional unit derived from an organic polyol or an organic polyisocyanate; BX represents a unit derived by reacting an organic polyisocyanate (X) with a sulfonate diol, phosphate diol, carboxylate diol, or quaternary ammonium diol; and a, b, and c are integers, a and b being greater than zero, and a, b, and c being selected so that the  $-(BX)-$  units make up no more than 40% by weight, preferably 0-20% by weight, of the hydrogel and the molar ratios of A:X:T in the final polymer are from 2:2:1 to 12:12:1, preferably 3:3:1 to 9:9:1.

Preferred dye films used herein to detect changes in pH are hydrogels having the aforementioned polymer units and also units of the formula  $-(DX)_d-$ , wherein D represents an azo dye reacted with X, an organic polyisocyanate, and d is an integer selected to provide 0.1% to 20% by weight in the hydrogel of the dye (D), preferably from about 1.0% to 10% by weight.

The structure and chemical composition of the hydrogels of this invention should be adjusted so that the final film is hydrated 20% to 90%, preferably 40% to 80%, with uniform water distribution and elastomeric properties.

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Special mention is also made of polyacrylamide hydrogels and polyurethane hydrogels in which the crosslinking component is a polysaccharide. Reactive azo dyes as disclosed herein may also be incorporated into these polymers to produce pH-indicating dye films.

Suitable polyalkylene glycols and thioglycols for use in preparing the hydrogels of the invention are poly(C<sub>2</sub>-C<sub>6</sub>)alkylene glycols and thioglycols such as polyethylene glycol, polypropylene glycol, polytetramethylene ether glycols, mixtures of such glycols and their corresponding thioglycols. The molecular weight of the polyalkylene glycols or thioglycols will be from 200 to 15,000, preferably from 600 to 3,000, to obtain the desired hydration. The percent hydration is defined as the weight percent of water in the completely hydrated film.

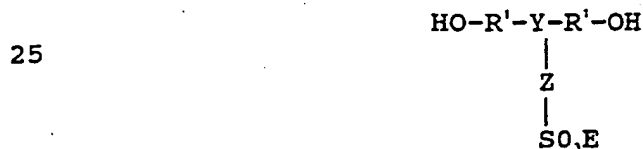
Suitable organic polyisocyanates useful for preparing the hydrogels have the general formula  $R(N=C=O)_x$ , wherein R is an aliphatic hydrocarbon radical of from 4 to 12 carbon atoms, a cycloaliphatic hydrocarbon radical of from 6 to 15 carbon atoms, or an aromatic or aromaliphatic hydrocarbon radical of 6 to 16 carbon atoms, and wherein x represents 2, 3 or 4. Examples of such polyisocyanates include tetramethylene diisocyanate, hexamethylene diisocyanate, 1,4-cyclohexane diisocyanate, 1,4-pentane diisocyanate, methylene bis(4-isocyanatoethyl cyclohexane), 1-isocyanato-3,3,5-trimethyl-5-iso-cyanato-methyl cyclohexane, 1,4-isocyanatomethyl cyclohexane, 1,4-isocyanatoethyl cyclohexane, 1,3-isocyanatomethyl cyclohexane, 1,3,5-triisocyanato-cyclohexane, diisocyanatopentylcyanomethine, 1,4-diisocyanobenzene, 2,6-diisocyanatotoluene, 2,6-diisocyanatotoluene, toluene diisocyanate (2,4- and 2,6-mixtures), 4,4'-

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dicyanodiphenylmethane, 4,4'-diisocyanodiphenyl propane (2,2), p-xylylene diisocyanate, and the like. Mixtures of such polyisocyanates can also be employed.

Optionally, heteroorganic diols and  
5 carboxylate diols can also be used in preparing the hydrogels of the present invention. Urethane hydrogel polymer units may be derived from reacting the aforementioned isocyanates with sulfonate diols, quaternary ammonium diols, carboxylate diols, and  
10 phosphate diols in order to give ionic strength to the hydrogel. The corresponding acids of such diols (i.e., sulfonic acid diols, etc.) may also be used. Where such diols are used, the hydrogel may include up to 40% by weight of the diol component, but preferably 20% or  
15 less. Such diol reactants readily copolymerize with the isocyanates to produce hydroxy-terminated polyurethanes, which react further with isocyanate-terminated prepolymers. This results in a single polymer population, as determined by gel permeation  
20 chromatography, with the diol components being distributed throughout the polyurethane chains.

Suitable sulfonate/sulfonic acid diols include compounds of the formula:



wherein R' may be the same or different bivalent  
30 aromatic or aliphatic radical of from 1 to 12 carbon atoms; Y is nitrogen, carbon, sulfur, silicon, or phosphorus, with any free valencies being taken by hydrogen or halogen atoms; Z is a direct bond or R'; and E is hydrogen, sodium, lithium, potassium, magnesium or  
35 calcium. Examples of such compounds include N,N-bis

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(2-hydroxyethyl)-2-N-methylaminoethane sulfonic acid, 1,4-butanediol-2-sulfonic acid, and salts thereof.

The polyurethane hydrogels of the present invention are prepared by reacting organic  
5 polyisocyanates with polyalkylene glycols and/or triols at an elevated temperature. Either isocyanate- or hydroxy-terminated polyurethane pre-polymers of different molecular weights can be produced in this manner depending on the stoichiometry. The polyols and  
10 polyfunctional isocyanates are used to control the crosslink density and degree of branching.

Hydroxy-functional dyes can be easily incorporated into the polyurethane network with either a hydroxy terminated polyurethane and multi-functional  
15 isocyanates, or alternatively, with isocyanate-capped polyurethane prepolymers during cure stage, thereby forming a water insoluble, but swellable hydrogel film. Thus, polyurethane hydrogels can be produced to meet various specifications by varying the stiochiometry of  
20 the isocyanate and polyol components as well as by including ionic diol groups and dye groups.

The reaction of isocyanates and polyols can be carried out either in bulk or solution. Aprotic solvents such as acetone, methyl ethyl ketone, ethyl  
25 acetate, cyclohexanone, DMF, dimethyl sulfoxide (DMSO) N-alkyl-pyrrolidone, and butyrolactone are excellent because they provide dissolution power, chemical stability and temperature control.

In producing prepolymers, the polymerization  
30 temperature can be varied from 20°C to 190°C depending on individual preparations and the presence of a solvent. The preferred temperature range is from 70°C to 130°C. An inert atmosphere is sometimes required to prevent undesirable side reactions.



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During the cure stage of the process, where a hydrogel film is being produced, the isocyanate and polyol reaction temperature is between 70°C to 140°C, preferably between 90°C to 110°C, for a desirable cure rate and film properties.

The polyaddition of isocyanate and polyols can also be carried out in the presence of either acidic or basic catalysts. Tertiary amines, carboxylic acids and metal salts are commonly used. Suitable tertiary amines include triethylamine, heptamethylisobiguanidine and triethylenediamine (1,4-diaza [2.2.2] bicyclooctane). The metal salt catalysts generally have greater catalytic power than the tertiary amines, and preferred examples include tri-n-butyltin acetate, di-n-butyltin diacetate and di-n-butyltin dilaurate. Formic acid is an excellent acid catalyst.

The following examples illustrate the preparation of hydrogels according to the present invention and are not intended to in any way limit the scope of the invention.

#### EXAMPLE 45

##### Polyurethane Prepolymer PEG1000/TDI/TMP:

Polyethylene glycol (PEG) (mol. wt. 1000, 100 g, 100 mmol) and trimethylolpropane (TMP) (3.35 g, 25 mmol) were dried under vacuum at 80°C for one hour. The vacuum was broken with nitrogen and toluenediisocyanate (TDI) (42.63g, 175 mmol) was added. The mixtures were allowed to react at 80°C for 1.15 hour. The polymer was stabilized with 100 ppm of benzoyl chloride. Dry dimethylformamide (DMF) (57.6 g) was added to make a 70% solution.

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EXAMPLE 46Polyurethane Prepolymer PEG600/TDI/TMP:

The polymer was prepared according to the procedure of Example 45 except a different grade of polyethylene glycol (mol. wt. 600, 84 g, 100 mmol) was used and no DMF was added.

EXAMPLE 47Polyurethane Prepolymer PEG10001TDI:

The polymer was prepared as in Example 45 except no TMP was added.

EXAMPLE 48Polyurethane Prepolymer PEG1000/CHDI:

The polymer was prepared as in Example 43 except cyclohexyldiisocyanate (CHDI) (29 g, 175 mmol) was used instead of TDI.

The viscosities and molar equivalents/gram of isocyanate for the isocyanate-terminated pre-polymers of Examples 45-48 are shown in Table 6.

TABLE 6

Example No.	Isocyanate (meg/gram)	Viscosity (cps)
45	0.47	2,260 (70%)
46	0.83	91,000
47	0.57	1,800 (70%)
48	0.58	31,600

EXAMPLE 49Polyurethane Prepolymer PEG1000/CHDI/TMP:

PEG1000 (100 g, 100 mmol) and TMP (3.35 g, 25 mmol) were dried under vacuum at 90°C for one hour. The vacuum was broken with nitrogen. CHDI (16.60 g, 100 mmol) was then added. The mixture was allowed to

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react at 90°C for 3 hours. After cooling, a solid polymer was isolated.

EXAMPLE 50Polyurethane Prepolymer PEG1000/TDI/TMP:

- 5           The polymer was prepared as in Example 49, except TDI was used. Enough DMF was added to make a 70% solution.

EXAMPLE 51Polyurethane Prepolymer PEG1000/TDI:

- 10           The polymer was prepared as in Example 50, except TMP was not used. Enough DMF was added to make a 70% solution.

EXAMPLE 52Polyurethane Prepolymer PEG600/TDI/TMP:

- 15           The polymer was prepared as in Example 50, except PEG600 was used. Enough DMF was added to make a 70% solution.

The viscosities and molar equivalents/gram of Examples 49-52 are shown in Table 7.

20

TABLE 7

Example No.	Hydroxyls (meg/gram)	Viscosity (cps, 70%)	No. Ave. Mol. Wt.
49	1.07	--	2553
50	1.32	1500	4220
25 51	1.21	1375	5600
52	1.18	1900	3830

EXAMPLE 53Ionic Prepolymer PEG1000/TDI:

- 30           PEG1000 (40.3 g, 40.3 mmol) was charged and dried in a reactor at 100°C vacuum for one hour. The

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vacuum was broken with dry nitrogen. TDI (9.61 g, 55.89 mmol) was added and the mixture reacted at 100°C for three hours until there was no TDI left, as shown by stabilization of the NCO peak on IR spectrophotometric analysis. 1,4-butanediol-2-sulfonic acid sodium salt (5.163 g, 26.87 mmol) in of DMSO (23.5 g) were added to the isocyanate-terminated prepolymer solution until the isocyanate peak in IR spectrum disappeared. The final polymer product showed one major population distribution with a number average molecular weight at 47,000.

#### EXAMPLE 54

##### Ionic Prepolymer PEG1000/TMP/TDI/MeBES:

N,N-bis(2-hydroxyethyl)-2-aminoethane sulfonic acid sodium salt (BES, Aldrich Chem. Co.) was methylated by the following procedure.

Dry sodium hydroxide (8.4 g, 210 mmol) was dissolved in 250 ml of methanol. BES (42.6 g, 200 mmol) and 200 mmol of dimethyl sulfate were added separately with vigorous agitation. The solution was constantly stirred over night. The methylated BES (MeBES) was filtered and recrystallized from water-methanol (m.p. 240°C; yield 70%).

PEG1000 (21.68 mmol) was dried under vacuum for an hour at 100°C in a reactor. The vacuum was broken with dry nitrogen. TDI (5.035 g, 28.91 mmol) was added and reacted at 100°C for two hours until no TDI remained, which yielded an isocyanate-terminated prepolymer. MeBES (3.285 g, 14.47 mmol) was charged to the reactor in DMSO (27.33 g) and reacted one hour until IR analysis showed no isocyanate peak. The resultant polymer showed one major population distribution of number average molecular weight at 11,903 (in DMF relative to PEG standard).

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The Dye Films

In the sensing elements of the present invention, a thin hydrogel film containing one or more of the aforementioned pH indicator azo dyes is prepared. By "thin hydrogel film" is meant a layer of about 0.5 to 50 microns thickness. In preferred embodiments, the film layers will be from about 1-10 microns, most preferably about 2-6 microns, in thickness.

When a thin film compounded with dye ("dye film") is hydrated it will change color instantaneously as a function of the acidity or basicity of the environment. Accordingly, such films are advantageously used as optical sensor elements for detecting any parameters which are a function of pH.

The dye film is formulated so as to provide, in thin layers of 0.5 to 50 microns, sufficient optical density and pH sensitivity to permit optical measurement, considering the light source, optical pathway and photodetector elements to be employed. For an optical reader using backscattered light such as described infra, suitable amounts of the dye component may range from about 0.1-15% by weight, based on the total weight of the dye-containing hydrogel polymer (non-swollen). Preferably the amount of dye will be about 1-10% by weight, most preferably about 3-8% by weight.

In addition to the dye film, optical sensing elements may include other components, depending on the overall configuration of the optical reader apparatus. For instance, in a preferred fluid analyzer embodiment described hereinafter, a backscattering optical pathway is employed, and, accordingly, the sensing elements of the analyzer will include a light scattering film

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adjacent to the dye film. In addition, to prevent interference from light which has passed the dye film and has been backscattered through the dye film again, the sensing element may also preferably include a light  
5 absorbing layer in order to render the optical measuring device insensitive to optical variation in the sensing cuvette interior. Furthermore, in order to make a pCO<sub>2</sub> sensing element, a gas permeable film is also juxtaposed between the dye film and the solution  
10 to be analyzed. Thus, the multilayer coatings can be fabricated to make pH and pCO<sub>2</sub> sensor devices.

The following examples illustrate the preparation of the dye films and pigment-containing films of the invention. No limitation of the scope of  
15 the invention is intended or should be inferred.

#### EXAMPLE 55

A dye film having the following formulation is prepared:

0.3 parts by weight B<sub>3</sub>-Tris (Example 22)  
20 7.2 parts by weight polyurethane hydrogel  
5.0 parts by weight dried DMF  
5.6% Dye Solution 60% Total Solids  
4% Dye Solids

The dye and DMF are mixed in a vial and  
25 sonicated for 40 minutes. Although a trace of material remains, the dye dissolves well. After centrifuging for 10 minutes, the solution is drawn off and filtered through a 0.2 micron syringe filter. 97% of the solution is recovered. After adjusting for the  
30 material which is lost, polyurethane hydrogel is mixed in by shaking and using a vortex mixer. The solution is centrifuged again for 10 minutes and filtered through a 5 micron syringe filter. 11.043 parts by weight of solution is recovered for an 88% overall

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recovery. A final centrifugation is performed for 10 minutes to remove any air bubbles.

Coating is begun 30 minutes after the hydrogel is added. Before coating, each disc polyester substrate on the vacuum chuck is washed and scrubbed with filtered MeOH. A final film thickness of 8-10 microns is required for an optical density of 0.5 at this dye loading. The discs are cured for 90 minutes at 110°C. Once out of the oven, the films are tacky but become fully cured on standing overnight. Unbound dye is removed by leaving the films for 5 minutes in DMF followed by 5 minutes in MeOH. Both solvents are filtered (0.45 micron). The DMF removes the majority of loose dye and dye impurities while the MeOH mainly serves to rinse out the DMF.

Table 8 gives examples of dye-containing hydrogels which were prepared and tested as pH-sensing materials.

TABLE 8

Dye	% Dye	% PEG		% Dye		Absorption	
		1000	Retained	pK <sub>a</sub>	Maxima, nm		
B <sub>5</sub> -DEA	5	16	57	-	510	650	
B <sub>5</sub> -MA	5	0	39	6.8	-	-	
B <sub>5</sub> -MeETA	5	0	40	6.38	-	-	
B <sub>5</sub> -TRIS	5	18	65	7.1	535	640	
A <sub>1</sub> -MA	5	20	56	9.2	530	640	
A <sub>2</sub> -TRIS	5	14	52	7.2	525	645	

## Notes:

- PEG = Polyethylene Glycol 1000      MA = Methylamine  
 MeETA = N-Methylethanolamine      DEA = Diethanolamine  
 TRIS = tris(hydroxymethyl)methylamine

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EXAMPLE 56Introduction of additional charged substituents:

A solution of 2,3-dibromopropanoamido azo dye (200 g), glycine (400 mg), and DABCO (20 mg) in 20 ml isopropanol/water (1:1) was stirred for 5 hours at 20°C at pH 8.5 to 9 and then precipitated by the addition of sodium chloride. The resulting dye was electrostatically bound to a polyelectrolyte-containing water-swelling polymer in a manner similar to the binding of azo dyes to yield a pH-indicating hydrogel. Polyacrylamide, which contains 5 to 25% polyethyleneimine (linked via acryloyl substituents, for example), took up the dissolved azo dye quickly from a solution. The dye remained bound even in dilute salt solutions.

15

EXAMPLE 57Incorporation of an acrylic azo dye into a polyacrylamide matrix:

A solution of B3-2-bromoacrylamide (200 mg), acrylamide/bisacrylamide (20:1) (50 mg) and acrylamide (50 mg) (FMC Corporation, Marine Colloids Division) in 3 ml water was dried and heated under nitrogen for 2 hours at 103°C. After washing, a water-swelling polymer containing a bound pH-indicating azo dye was obtained.

25

EXAMPLE 58Preparation and binding of an azo dye which contains an aromatic amino group:

The diazonium sulfate converted from 2-bromo-4,6-dinitroaniline (550 mg) in Example 15 was added to a slurry prepared from 4-amino-5-hydroxy-1-naphthalene-sulfonic acid (500 mg), water (25 ml), ice (10 g) and H<sub>2</sub>SO<sub>4</sub> (1.5 ml). After completion of the reaction at 20°C, the dye was precipitated by the addition of



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sodium chloride and subsequently dried. Up to 100 mg of dye was dissolved in dry dimethylsulfoxide. After the addition of the isocyanate terminated polyurethane hydrogel, the dye became bound to the polyurethane. A pH-indicating hydrogel was formed.

#### EXAMPLE 59

Preparation of a polysaccharide covalently bound to a pH-indicating azo dye:

A solution of soluble starch (2.0) in 10 ml water containing 2%  $K_2HPO_4$  and 2.5% sodium borate is stirred with a 2,3-dichloropropano-amido-substituted azo dye (50-500 mg) for 1 hour at 60°-80°C. Unreacted dye and reagents may be removed by dialysis or gel filtration. The resulting polymeric pH-indicating material is lyophilized and subsequently incorporated in a variety of hydrogel-forming materials by simply admixing before curing. Examples of hydrogel-forming materials include isocyanate-terminated polyurethane prepolymers. The polysaccharide acted as the cross-linking reagent. It is also possible to form a hydrogel by cross-linking the dye-containing polysaccharide using readily available di- or polyfunctional reagents.

#### EXAMPLE 60

PEG1000/TDI/B.MeETA:

PEG1000 (2.53 g) was dried in a reactor under vacuum at 100°C for one hour and the vacuum was broken with dry nitrogen and TDI (0.551 g) was added. The mixture was allowed to react for two hours at 100°C to produce an isocyanate-terminated prepolymer. B.MeETA (1g) in dry DMSO (9g) was charged to the reactor. The reaction was completed in thirty minutes as evidenced by IR spectra. The number average molecular weight of

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the hydroxy-terminated dye prepolymer product was at 13,000 with a broad molecular weight distribution and hydroxyl content of 0.32 meq./gm.

In a control experiment where all reactants were initially mixed together, the materials produced exhibited a molecular weight distribution after gel permeation chromatography indicating two distinct polymer species.

In another control experiment, where the TDI and B<sub>2</sub>MeETA were allowed to react for 30 minutes before adding polyethylene glycol, the resultant material also showed a bimodal molecular distribution.

#### EXAMPLE 61

##### OH terminated/Dye (5%)/TDI formulation:

A hydroxy-terminated polyurethane prepared according to the techniques of the foregoing examples, using PEG1000, TDI and TMP (6.17 g, 70%) was mixed under nitrogen atmosphere with B<sub>2</sub>MeETA (0.257 g) and TDI (0.56 g), with enough DMF to make a 55% solution at room temperature. The viscous solution was spin coated on a Mylar disk to form a 10 micron-thick film, followed by curing at 110°C for two hours. Any unreacted or loosely bonded dye was leached out in DMF. The dye-hydrogel film reached equilibrium hydration of 65% in less than one minute. It changed color from red to blue reversibly on contacting different pH solutions. The pK<sub>a</sub> of the film was 7.05.

The above procedures were used to prepare films using different dyes. The resulting films are described in Table 9, infra.

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TABLE 9

	<u>Dye</u>	<u>% Dye</u>	<u>% PEG</u> <u>1000</u>	<u>% Dye</u> <u>Retained</u>	<u>pK<sub>i</sub></u>	<u>Absorption</u> <u>Maxima, nm</u>	
	B <sub>3</sub> -DEA	6.5	67.5	78	7.92	530	650
5	A <sub>3</sub> -DEA	5	68.5	76	9.54	-	-
	A <sub>3</sub> -DEA	5	68.5	-	6.08	530	655
	B <sub>3</sub> -BHPA	5	68.5	85	7.13	540	645
	A <sub>3</sub> -BHPA	4	70.2	71	9.32	530	645
	A <sub>3</sub> -BHPA	3.7	70.2	90	7.12	540	645
10	A <sub>11</sub> -BHPA	3.6	70.3	87	7.42	540	635
	A <sub>17</sub> -BHPA	3.9	70.1	81	7.92	540	650

EXAMPLE 62OH-terminated/Dye (5%) Desmodur IL formulation:

A dye hydrogel was formulated and cured  
 15 following the procedures of Example 61, except that a  
 trifunctional isocyanate, Desmodur IL (Mobay  
 Chemicals), was used. The film had only 31% hydration  
 and a pK<sub>i</sub> of 6.51.

EXAMPLE 6320 OH terminated/Dye (8%)/Desmodur IL Formulation:

A dye hydrogel was prepared following the  
 procedures of Example 61, except the amount of dye was  
 adjusted to make up 8% by weight of the total  
 formulation. The film had 56% hydration and a pK<sub>i</sub> of  
 25 6.74.

EXAMPLE 64OH terminated/Dye (5%)/TDI/Desmodur IL:

The hydroxy-terminated polyurethane  
 prepolymer of Example 50, the diol dye B<sub>3</sub>MeETA, and a  
 30 mixture of TDI-Desmodur IL in a ratio of 30%-70% (mole)  
 were reacted using the same procedures as in

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Example 60. The resultant film had a 72% hydration and a  $pK_a$  of 6.70.

#### EXAMPLE 65

##### OH-terminated ionic/Dye (5%)/Desmodur IL:

5           The hydroxy-terminated ionic polyurethane prepolymer from Example 54 (3.30), B<sub>2</sub>MeETA dye (0.119 g), and Desmodur IL (1.479 g) were mixed well and the solution was spin-coated on Mylar to form a 6.5 micron film. The film had a 41% hydration and a  $pK_a$  of  
10 6.80.

#### EXAMPLE 66

##### Isocyanate-terminated/PEG1000/Dye (5%):

          The isocyanate-terminated polyurethane hydrogel (1.17 g) PEG1000 (0.26 g) and B<sub>2</sub>-Tris dye  
15 (0.075 g) (5% by weight) are mixed with DMF (1.426 g). The solution is spin-coated and cured on Mylar to form a 5 micron film. The film has a 65% hydration and a  $pK_a$  of 7.07, and also possesses acceptable optical density.

#### EXAMPLE 67

##### Isocyanate-terminated/Dye (4%):

          B<sub>2</sub>-Tris dye (0.3 g, 4% by weight) and the isocyanate-terminated polyurethane hydrogel (7.2 g) are mixed in dry DMF (5 g). The solution is spin-coated and cured as in Example 48. The film had a 43%  
25 hydration and a  $pK_a$  of 7.4.

#### EXAMPLE 68

##### Isocyanate-terminated/Dye (8%):

          B<sub>2</sub>-Tris dye (0.25 g, 8% by weight) and the isocyanateterminated polyurethane hydrogel (2.875 g)  
30 are mixed in DMF (2.66 g). The solution is coated and

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cured as in Example 65. The film had a 30% hydration and a  $pK_a$  of 7.3. This is a good film for using as a sensor.

#### EXAMPLE 69

##### 5 Isocyanate-terminated/Dye (10%):

$B_2$ -Tris dye (0.25 g, 10% by weight) and the isocyanateterminated polyurethane hydrogel (0.5 g) are mixed in dry DMF (2.25 g). The solution is spin coated as in Example 65, to form a 4 micron film. The film  
10 had a  $pK_a$  of 6.72.

#### EXAMPLE 70

##### OH terminated/Dye bonded/TDI/Desmodur IL:

The dye-containing hydroxy-terminated pre-polymer of Example 59 (0.493 g) TDI (0.068 g) and  
15 Desmodur IL (0.052 g) were mixed well. The solution was spin-coated and cured as in Example 65. The film had a 68% hydration and a  $pK_a$  of 6.72.

#### EXAMPLE 71

##### White reflective coating:

20 A pigment-containing hydrogel film may be prepared by mixing a white pigment (Rutile, a crystal form of titanium dioxide, 79.2 g), a hydroxy-terminated polyurethane prepolymer (28.3 g) and cyclohexanone (42.5 g), and dispersing the solution in a ball mill to  
25 prepare a mill base. The solution is then compounded with polyurethane (40 g), cyclohexanone (64 g) and TDI (5.42 g) along with a small amount of catalyst such as di-n-butyltin dilaurate and triethylenediamine (1,4-diaza[2.2.2]bicyclooctane). The resultant coating mix  
30 is used to deposit a white reflective layer by spin coating.

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EXAMPLE 72Black absorptive coating:

A pigment-containing hydrogel film may be prepared by mixing a black pigment (carbon black, 28 g),  
5 polyurethane prepolymer (23 g) and cyclohexanone (99 g), and dispersing the solution in a ball mill to prepare a mill base. The mill base is compounded with polyurethane (55 g), cyclohexanone (120 g) and TDI (7 g), along with a small amount of catalyst as  
10 mentioned in Example 71. The resultant mix is used to deposit a black absorptive layer by spin coating.

The Preparation of Multilayer Sensing Elements

To fabricate a multilayer sensing element, a pH-indicator dye film and any other necessary layers  
15 may be deposited directly on the interior surface of a cuvette cell into which fluids to be analyzed will be introduced; however, for ease of fabrication and replacement, it is much preferred to deposit the layers on an optically-clear support sheet, such as a 5-mil  
20 thick polyester film (e.g., Melinex film, ICI Americas Inc., Wilmington, Delaware or Mylar, E. I. DuPont de Nemours, Inc.).

It is important that the hydrogel polymer maintain its structural integrity when it is loaded  
25 with indicator dyes, pigments or other substances, so that it possesses the appropriate optical and chemical properties in the pH range of interest.

For fluid analyzer apparatus which measures reflected light which has passed through the dye film,  
30 a thin reflective film is deposited over the dye film. The reflective film comprises a hydrogel and at least one pigment having a higher refractive index than water, preferably titanium dioxide. The hydrogel may be

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the same or different than that of the dye film layer, and in addition to the polyurethane hydrogels discussed above, special mention is made of hydrogels based on polyacrylamides, many of which maintain especially high water contents even with high pigment loadings.

A further pigmented film advantageously applied over the reflective film layer is a light absorptive film. This film is similar to the reflective film, except that its pigment absorbs electromagnetic radiation rather than reflecting it. Carbon black is the most preferred pigment for the light absorptive film.

Several methods for compounding and casting the dye films and pigment-containing films will be apparent to those skilled in the art. In one method, appropriate amounts of the components and a solvent (such as DMF) are weighed into a vial. The dye is then thoroughly dissolved by sonication, stirring or heating. Undissolved solids are removed by centrifugation. At this point the solution is drawn off and passed through a filter, e.g., 0.2 micron, to eliminate suspended particles. The solution is weighed to correct for the material lost in the filtration and a weighed amount of prepolymer is added. Cross-linking agents may also be added. The total percentage of solids in these formulations is usually about 60%, which gives the solution a sufficiently high viscosity for good coatability, but is not so high as to preclude it from spin coating films of only a few microns thickness. The prepolymer is mixed in by stirring or shaking. The solution is again centrifuged to make subsequent filtration through a 1 micron membrane easier. This step removes precipitated material and solids resulting from introduction of the prepolymer. A final centrifugation removes any air bubbles.

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In order to prepare thin films, the hydrophilic polyurethane solutions thus formulated (e.g., with pigment or dye) are coated either onto an optically clear substrate or directly into the sensing  
5 cells of the cuvette (discussed infra), and then cured. Preferably spin coating methods are used.

The viscosity of the coating solution is critical to the coating processes and the film quality. If it is too viscous, the desired thickness cannot be  
10 obtained and the film quality is poor and nonuniform. On the other hand, if the solution viscosity is too low, the film would be too thin and contain pin-holes. Viscosity is a function of polymer molecular weight, total solids, solvent type, temperature, and the  
15 presence of other ingredients which may react with the polymer components. The viscosity also varies according to the processing equipment. For thin film spin coating (0.5 to 50 microns), the desired viscosity is generally in the range of about 100 to 1,000  
20 centipoise.

Spin coating is a complex process, and there are numerous interactive variables which may have an effect on the results. These variables include spinning speeds, spinning times, spinning acceleration,  
25 viscosity, viscoelasticity of the coating solution, temperature, solvent evaporation (adiabatic or nonadiabatic), substrate topology, surface tension, interfacial surface tension, and dispersion quantity. Although simplified mathematical models have been  
30 developed (see, e.g., ACS Polymeric Material Science and Engineering, Vol. 55, pp. 99-101), they are of limited value in avoiding at least some experimentation in practical spin coating applications.

In spin coating, a small amount of dye-  
35 polymer solution is dispensed in the center of a



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stationary substrate. The substrate is rapidly accelerated centrifugally to a final rpm where it is held for several seconds. After coating, the dye films are cured, e.g., in a forced air oven. Depending on  
5 the formulation, complete curing is usually achieved in two hours at 90 to 110°C.

A "reflective" (i.e., backscattering) film formulation may be prepared by blending a pigment such as titanium dioxide (e.g., Kemira rutile) with the  
10 polymer used to make the reflective layer, with a solvent if desired. Pigment loading determines how thick the layer needs to be to give satisfactory reflective properties, keeping in mind that thicker layers lead to longer dynamic response times.  
15 Generally, however, for thin reflective films of about 3-5 microns thickness, pigment loadings of about 50-60% by weight are sufficient.

The pigment-containing formulations are milled for a long period, e.g., 1-10 days depending on  
20 such factors as pigment loading, type of mill, presence of a solvent, etc., until a mill base having the desired particle size and desired viscosity is achieved. The mill base is then formulated with additional prepolymers (including polyfunctional  
25 crosslinking components) stirred, and filtered (e.g., 5 microns) before coating over the dye film layer.

A non-reflective, absorptive film may be prepared in much the same manner as the reflective film. When deposited over the reflective film, the  
30 absorptive layer serves an optical decoupling function, making the measurement of the dye color immune to variations in the reflectivity and/or absorptivity of electromagnetic radiation of the fluid sample to be analyzed. Carbon black (e.g., Raven 1040, Columbian  
35 Chemical) is the preferred pigment. By blending carbon

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black with a hydrophilic polymer, a cured polymer layer can be made which acts as an optical barrier. The absorptive nature of carbon black is such that a 1 micron layer with only a 25% pigment loading by weight is sufficiently opaque.

For pCO<sub>2</sub> measurement, an additional film is used in the multilayered sensing element. A gas-separating membrane is coated over the non-reflective, absorptive film. This allows for effective diffusion rates of water vapor and CO<sub>2</sub> gas, while liquid water and ions are excluded. Alternatively, the membrane can be placed between the dye film and the reflective film which results in shorter response times to pCO<sub>2</sub> changes.

Three additional processing steps distinguish the pCO<sub>2</sub> sensing element from the pH sensing element. These steps all occur between the washing of the dye layer and the coating of the white layer.

The pCO<sub>2</sub> sensor functions by changes in pH, caused by shifts in the pCO<sub>2</sub>-carbonic acid equilibrium, producing differences in the dye absorbance. For maximum sensitivity the population of dye molecules should first be converted to their anionic form before being sealed in their environment with the gas-separating membrane. Soaking the dye-polymer films in aqueous solutions of pH greater than the pK<sub>a</sub> of the dye can deprotonate the desired amount of dye. Two aspects of this step are important: The percentage of dye converted to its salt must be very reproducible, and the process cannot leave behind any species which interfere with protonation.

The films are soaked in buffer solution having high pH (e.g., pH 9-10) which has preferably been filtered (e.g., 0.45 micron) for cleanliness. Before the next step, the films are allowed to air dry completely.

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By allowing only neutral species to pass, the gas-permeable layer, preferably a silicone membrane, isolates the dye layer from ionic species, specifically hydronium. Polydimethylsiloxane is most preferred for the membrane material because of its high permeability to carbon dioxide and water. Physically, the membrane needs to be thin enough for an acceptable response time while having good integrity so that no bulk fluid can pass through. Like the other films, the silicone membrane is made by spin coating a solution of silicone polymer in a suitable solvent. Silicone elastomers are diluted to an appropriate percent solids level for spin coating thin films. Xylene and polydimethylsiloxane solvents with a b.p. between 110-150°C are used. The silicone products Petrarch Systems SE and Dow Corning 3140 are especially suitable to fashion membranes. As sold, Petrarch SE is 35% solids in a naphtha solvent. After dilution, the silicone solution is mixed by shaking and then is filtered through at least a 1 micron syringe filter to remove particulates. Centrifugation for ten minutes removes any air bubbles. The choice of spin speed depends on the desired thickness and % solids. When coated, the substrate is first flooded with silicone solution to assure that the entire surface is wetted. Many commercial silicones (e.g., Petrarch SE) fully cure in a matter of hours upon exposure to moisture.

Cured silicone elastomers are known for their inertness and low surface energy. Coating or painting silicone materials is customarily difficult because of this very low surface energy. Since there is no surface pretreatment, the coating quality is very poor when the reflective film is spincoated onto the silicone membrane. The polymer solution dewets to such an extent that the entire surface can easily become

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uncovered. Plasma activation of the silicone surface may be used to both increase the wettability so that acceptable coatings can be made and to enhance the adhesion to these subsequent layers. Plasma activation  
5 by exposure to radio frequency discharge of a selected gas is a chemically complex process and will not be discussed here in detail.

The Sensing Elements in a Body Fluid Analyzer

A preferred body fluid analyzer,  
10 incorporating the hydrogels and dyes already disclosed, will be described with reference to the drawings. It will be understood that the hydrogels and dyes, dye films and sensing elements prepared therefrom, may be employed for purposes radically different the uses  
15 described herein design without departing from the scope of this invention. Similarly, the following description illustrates a preferred embodiment and is not provided to in any way limit the present invention. The body fluid analyzer described hereinafter is only  
20 one example of a multitude of possible designs which will be apparent to those familiar to this art.

Referring to Figure 1, the body fluid analyzing apparatus is comprised of several linked sub-units, shown as separated structures (1, 2, 3, 5).  
25 Such sub-units could, of course, also be united in a single housing but are drawn as discrete units for ease of explanation.

A monitoring unit 1 is provided which is capable of data signal evaluation and data display.  
30 Depending on the particular design of the analyzer, its power requirements, and the particular optical measuring equipment used, a signal preamplification unit 2 may be provided to boost the photoreceptor data signal corresponding to the optical measurement of pH

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in the sensing cells (described infra). A cuvette housing 3 is provided incorporating optical measurement components including an electromagnetic radiation source ("light source") and photodetectors (not shown) aligned so as to obtain color measurements of samples placed in a sensing cuvette 5 and having a slot 4 adapted for receiving the sensing cuvette 5. The sensing cuvette 5 is preferably equipped with tubing adapters 6 which permit introduction of a fluid to be measured into an interior channel (not shown) running through the cuvette 5 where the fluid contacts the sensing elements (not shown) of the apparatus. Through optically clear windows 7 and 8, located on one side of the cuvette 5, color changes reflecting pH and pCO<sub>2</sub> changes can be measured by optical measuring components in cuvette housing 3.

The cuvette has one or more sensing cells, each in open communication at one end with the channel in the cuvette interior and being closed at the other end by optically clear windows 7 and 8, which windows 7 and 8 come into alignment with the measuring optics of the cuvette housing 3 when the cuvette 5 is properly inserted in the slot 4.

Referring to Figure 2, a cross-sectional view of a possible sensing cuvette 5 is shown. Optically clear windows 7 and 8 are shown forming the closed ends of two sensing cells 13 and 13A, into which multilayered sensing elements have been inserted. In the diagram shown, two sensing cells are shown, having sensing elements for optical measurement of pH (13) and pCO<sub>2</sub> (13A) however, it will be seen that numerous additional cells may be fabricated in the body of the cuvette 5 (for pH, pCO<sub>2</sub>, or other measurements) depending on design preferences and the requirements of the practitioner.

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As seen in Figure 2, the sensing cuvette 5 is provided with inlet and outlet tubing 6 providing access to a channel 15 which provides a passageway through the cuvette 5. Sensing cells 13 and 13A open on the channel 15 to afford contact of the multilayered sensing elements with any fluid samples introduced into the channel 15. The sensing elements are comprised of a dye film 9 and other layers which adapt the sensing elements to the particular optics and design of the cuvette 5 and the analyzer as a whole. In the embodiment pictured, the sensing elements are adapted to permit optical measurement of light transmitted and backscattered through the dye film 9, which dye film changes color as a function of pH.

The sensing cell 13 adapted to detect pH of a solution passing through the channel 15 features an optically clear window 7 which will come into line with the particular optic components of the cuvette housing (item 3 in Fig. 1). The pH-indicator dye film 9 may be coated directly on the window 7, but preferably the film 9 (and other layers) will be deposited on an optically clear substrate 16, such as polypropylene or a polyester film (e.g., 10 mil Mylar). Many suitable substrates will suggest themselves to the practitioner. In the cell 13, a dye film 9 is deposited (e.g., spin coated) on a clear polymeric substrate 16. A reflective film 10 is deposited on the dye film 9, and an absorptive film 11 is deposited over the reflective film 10. In the sensing cell 13A, adapted to the detection of  $pCO_2$ , the optically clear substrate 16 adjacent the optically clear window 8 is coated with a dye film 9, a reflective film 10 and an absorptive film 11, and also a gas permeable silicone layer 12. The gas-separating film 12 may be positioned elsewhere, for instance between layers 9 and 10, if desired.

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The inner dimensions of the cuvette 5 are preferably adapted to minimize the volume of fluid needed for analysis while providing sufficient volume to ensure contact with the sensing elements and completion of the protonation reaction with the indicator dyes. For illustration, the cuvette may be about 40 mm high and about 20 mm wide, and the channel 15 may be about 9 mm in diameter. In operation, water and ions in the fluid diffuse freely through the hydrogel layers of the sensing cell 13 and contact the pH indicator dye in the dye film 9, which undergoes a shift of the protonation equilibrium, resulting in a shift in the wavelength of absorbance of light passing through the dye film. In the pCO<sub>2</sub> sensing cell 13A, water vapor and CO<sub>2</sub> gas from the fluid diffuse through the gas-separating membrane 12. The water vapor hydrates the dye film in the presence of the CO<sub>2</sub> gas, resulting in the formation of carbonic acid which subsequently dissociates, altering the protonation equilibrium of the dye.

In Figure 3, the cuvette 5 is shown in relation to a light source 18 and an optical receptor 20, which would be contained in the analyzer housing (3 in Fig. 1). The light source 17 directs light (arrowed lines) toward the sensing cell 13. Light passes through the window 7, the clear film substrate 16 and the dye film 9, and then is backscattered off the reflective film 10. Photoreceptor 21 is positioned to accept the returned light emanating from the cell 13. The backscattered light differs from the source light directed as a function of the color of the dye film, and therefore gives an indication of the pH of the solution passing through the cuvette channel 15 in contact with the cell 13. The photoreceptor 20 emits an electronic data signal which is passed along to

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preamplification and data evaluation equipment  
(Fig. 1).

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Table 1

Example No.	Dye Name	Diazonium Comp.	Coupling Comp.	Yield	Color acid	Color base	pKa	Lambda Max, nm acid base	Isobestic Point
4	B <sub>2</sub> -2-Br-acrylamide	2-Br-4,6-diNO <sub>2</sub> -phenyl	4-(NHCOBrCH <sub>2</sub> ) 5-OH-1-SO <sub>3</sub> H	24%	red	blue	6.8	525 620	555
5	A <sub>1</sub> -2-Br-acrylamide	2,4-diNO <sub>2</sub> -phenyl	4-(NHCOBrCH <sub>2</sub> ) 5-OH-1-SO <sub>3</sub> H	25%	red	blue	8.8	530 610	550
6	A <sub>1</sub> -2-Br-acrylamide	2,Cl-4,6-diNO <sub>2</sub> -phenyl	4-(NHCOBrCH <sub>2</sub> ) 5-OH-1-SO <sub>3</sub> H	43%	red	blue	6.7	525 620	555
7	A <sub>1</sub> -2-Br-acrylamide	2,6-diNO <sub>2</sub> -4-CF <sub>3</sub> -phenyl	4-(NHCOBrCH <sub>2</sub> ) 5-OH-1-SO <sub>3</sub> H	18%	red	purple-blue	7.3	515 580	535
8	A <sub>1</sub> -2-Br-acrylamide	2,6-diNO <sub>2</sub> -phenyl	4-(NHCOBrCH <sub>2</sub> ) 5-OH-1-SO <sub>3</sub> H	61%	red	purple	8.0	520 545	530
9	A <sub>1</sub> -2-Br-acrylamide	2,4-diNO <sub>2</sub> -6-CF <sub>3</sub> -phenyl	4-(NHCOBrCH <sub>2</sub> ) 5-OH-1-SO <sub>3</sub> H	33%	red	blue	5.8	520 625	550
10	A <sub>1</sub> -2-Br-acrylamide	2,6-diNO <sub>2</sub> -4-CONH <sub>2</sub> -phenyl	4-(NHCOBrCH <sub>2</sub> ) 5-OH-1-SO <sub>3</sub> H	27%	red	purple-blue	7.3	520 590	540

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Table 2

Diazonium Comp.	Coupling Comp.	Yield	Color acid	Color base	pKa
4-NO <sub>2</sub> -1-naphthyl	4-(NHCOCBrCH <sub>2</sub> ) 5-OH-1-SO <sub>3</sub> H	67%	red-purple	purple blue	10.0
4-Cl-2-CN-phenyl	4-(NHCOCBrCH <sub>2</sub> ) 5-OH-1-SO <sub>3</sub> H	84%	orange	purple	high
4-Cl-2-CN-phenyl	4-(NHCOCBrCH <sub>2</sub> ) 5-OH-1-SO <sub>3</sub> H		red	red	none
2-CN-4-NO <sub>2</sub> -4-NO <sub>2</sub> -phenyl	4-(NHCOCBrCH <sub>2</sub> ) 5-OH-1-SO <sub>3</sub> H	37%	red	purple	7
2-CN-4-NO <sub>2</sub> -4-NO <sub>2</sub> -phenyl	4-(NHCOCBrCH <sub>2</sub> ) 5-OH-1-SO <sub>3</sub> H		orange	blue	6.5
5-NO <sub>2</sub> -thiazolyl	4-(NHCOCBrCH <sub>2</sub> ) 5-OH-1-SO <sub>3</sub> H	37%	orange	blue	4.0
6-NO <sub>2</sub> -benzo-thiazolyl	4-(NHCOCBrCH <sub>2</sub> ) 5-OH-1-SO <sub>3</sub> H	15%	orange	blue	6.0
5-(4-NO <sub>2</sub> -phenyl-S(O) <sub>2</sub> )thiazolyl	4-(NHCOCBrCH <sub>2</sub> ) 5-OH-1-SO <sub>3</sub> H	60%	orange	blue	5.0
2-NO <sub>2</sub> -4-benzoyl-phenyl	4-(NHCOCBrCH <sub>2</sub> ) 5-OH-1-SO <sub>3</sub> H	26%	red	purple	11.0

Table 1

Example No.	Diazonium Comp.	Coupling Comp.	Yield	acid	Color base	Color pKa
12	2,4-dinitro-phenyl	4-(NHCOCBrCH <sub>3</sub> ) 5-OH-2,7-diso <sub>3</sub> H	7%	orange	blue	6.0
13	4-NO <sub>2</sub> -naphthyl	4-(NHCOCBrCH <sub>3</sub> ) 5-OH-2,7-diso <sub>3</sub> H	13%	purple	blue	11.0
14	2-Cl-4,6-dinitro-phenyl	4-(NHCOCBrCH <sub>3</sub> ) 5-OH-2,7-diso <sub>3</sub> H	29%	orange	blue	6.0
15	2-Cl-4,6-dinitro-phenyl	4-(NHCOCBrCH <sub>3</sub> ) 5-OH-2,7-diso <sub>3</sub> H	26%	red	purple	8.5
15	2-Br-4,6-dinitro-phenyl	4-(NHCOCBrCH <sub>3</sub> Br) 5-OH-2,7-diso <sub>3</sub> H		orange	blue	6.0
16	2-Br-4,6-dinitro-phenyl	4-(NHCOCBrCH <sub>3</sub> Br) 5-OH-2,7-diso <sub>3</sub> H		red	purple	9.0
16	2-CN-4-NO <sub>2</sub> -phenyl	4-(NHCOCBrCH <sub>3</sub> ) 5-OH-2,7-diso <sub>3</sub> H	37%	orange	purple	5.0
	2-CN-4,NO <sub>2</sub> -phenyl	4-(NHCOCBrCH <sub>3</sub> ) 5-OH-2,7-diso <sub>3</sub> H		red	purple	5.0

Table 4

Dye Name	Diazonium Comp.	Coupling Comp.	acid	Color base	Color pKa
B <sub>1</sub> -2,3-diCl propionamide	2-Br-4,6-diNO <sub>2</sub> - phenyl	6-(NHCOCClHCClH <sub>2</sub> ) 4-OH-2-SO <sub>3</sub> H	orange	blue	7.0
B <sub>1</sub> -2,3-diCl propionamide	2,4-diNO <sub>2</sub> -phenyl	6-(NHCOCClHCClH <sub>2</sub> ) 4-OH-2-SO <sub>3</sub> H	orange	blue	8.0
B <sub>1</sub> -acrylamide	2-Cl-4,6-diNO <sub>2</sub> - phenyl	6-(NHCOCHCH <sub>2</sub> ) 4-OH-2-SO <sub>3</sub> H	orange	blue	
B <sub>1</sub> -acrylamide	2-MeO-4-NO <sub>2</sub> -phenyl	6-(NHCOCHCH <sub>2</sub> ) 4-OH-2-SO <sub>3</sub> H	red- purple	purple	10.0
B <sub>1</sub> -2,3-Br <sub>2</sub> propionamide	2-Br-4,6-diNO <sub>2</sub> - phenyl	8-(NHCOCHBrCH <sub>2</sub> Br) -4-OH-2-SO <sub>3</sub> H	red- purple	blue	
B <sub>1</sub> -2,3-Br <sub>2</sub> propionamide	2-Br-4,6-diNO <sub>2</sub> - phenyl	7-(NHCOCHBrCH <sub>2</sub> Br) -4-OH-2-SO <sub>3</sub> H	yellow	blue	6.5
B <sub>1</sub> -2,3-Br <sub>2</sub> propionamide	2-Br-4,6-diNO <sub>2</sub> - phenyl	6-(NHCOCHBrCH <sub>2</sub> Br) -7-OH-2-SO <sub>3</sub> H	yellow	purple	9.0

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Table 5

Dye Name	Diazonium Comp.	Coupling Comp.	Yield	Purity	Color acid	Color base	pKa	Lambda Max, nm acid-base	Isobestic Point
B <sub>3</sub> -2-Br-acetamide	2-Br-4,6-dino <sub>2</sub> -phenyl	4-(NHCOCH <sub>3</sub> Br) 5-OH-1-SO <sub>3</sub> H	55%	92%	red	blue	7.50	560 601	none
B <sub>3</sub> -2-Br-acetamide	2-Br-4,6-dino <sub>2</sub> -phenyl	4-(NHCOCH <sub>3</sub> Br) 5-OH-1-SO <sub>3</sub> H	63%	100%	red	blue			
A <sub>3</sub> -2-Br-acetamide	2-Cl-4,6-dino <sub>2</sub> -phenyl	4-(NHCOCH <sub>3</sub> Br) 5-OH-1-SO <sub>3</sub> H	70%		red	blue			
A <sub>3</sub> -2-Br-acetamide	2,4-dino <sub>2</sub> -6-CF <sub>3</sub> -phenyl	4-(NHCOCH <sub>3</sub> Br) 5-OH-1-SO <sub>3</sub> H	12%	93%	red	blue	6.00	insol 607	none
A <sub>3</sub> -2-Br-acetamide	4,6-dino <sub>2</sub> -2-SO <sub>3</sub> -phenyl Na <sup>+</sup>	4-(NHCOCH <sub>3</sub> Br) 5-OH-1-SO <sub>3</sub> H	48%		red	blue	7.20	512 608	550
B <sub>3</sub> -2-DEA-acetamide	2-Br-4,6-dino <sub>2</sub> -phenyl	4-(NHCOCH <sub>3</sub> Br) 5-OH-1-SO <sub>3</sub> H	83%	94%	red	blue	6.70	512 610	545
A <sub>3</sub> -2-DEA-acetamide	2-Cl-4,6-dino <sub>2</sub> -phenyl	4-(NHCOCH <sub>3</sub> Br) 5-OH-1-SO <sub>3</sub> H	17%						
A <sub>3</sub> -2-DEA-acetamide	2,4-dino <sub>2</sub> -6-CF <sub>3</sub> -phenyl	4-(NHCOCH <sub>3</sub> Br) 5-OH-1-SO <sub>3</sub> H	59%	98%	red	blue	5.80	514 611	540
A <sub>3</sub> -2-DEA-acetamide	4,6-dino <sub>2</sub> -2-SO <sub>3</sub> -phenyl Na <sup>+</sup>	4-(NHCOCH <sub>3</sub> Br) 5-OH-1-SO <sub>3</sub> H	40%	94%	red	blue	7.50	513 619	545
B <sub>3</sub> -succinamide	2-Br-4,6-dino <sub>2</sub> -phenyl	4-(NHCOCH <sub>2</sub> CH <sub>2</sub> -COOH) 5-OH-1-SO <sub>3</sub> H	44%	86%	red	blue	7.00	519 610	

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Table 6

Dye Name	Diazonium Comp.	Coupling Comp.	Yield	Purity	Color acid	Color base	pKa	Lambda Max, nm acid base	Isobestic Point
B <sub>3</sub> -BHPA	2-Br-4,6-d-INO <sub>2</sub> -phenyl	4-(NH-BHPA) 5-OH-1-SO <sub>3</sub> H	37%	96%	red	blue	6.50	518 608	540
A <sub>2</sub> -BHPA	2-Cl-4,6-d-INO <sub>2</sub> -phenyl	4-(NH-BHPA) 5-OH-1-SO <sub>3</sub> H	44%	92%	red	blue	6.40	518 608	545
A <sub>3</sub> -BHPA	2,4-d-INO <sub>2</sub> -6-CF <sub>3</sub> -phenyl	4-(NH-BHPA) 5-OH-1-SO <sub>3</sub> H	2%	99%	red	blue	5.70	516 610	540
A <sub>4</sub> -BHPA	2,6-d-INO <sub>2</sub> -4-CONH <sub>2</sub> -phenyl	4-(NH-BHPA-SO) 5-OH-1-SO <sub>3</sub> H	35%	38%	red	purple	7.00	516 581	530
A <sub>6</sub> -BHPA	4,6-d-INO <sub>2</sub> -2-SO <sub>3</sub> -phenyl Na <sup>+</sup>	4-(NH-BHPA-SO) 5-OH-1-SO <sub>3</sub> H	30%	83%	red	blue	7.40	515 613	550
A <sub>9</sub> -BHPA	4,6-d-INO <sub>2</sub> -2-CONH <sub>2</sub> -phenyl	4-(NH-BHPA) 5-OH-1-SO <sub>3</sub> H	6%	97%	red	purple	9.30	525 579	560
A <sub>10</sub> -BHPA	2-CN-4-NO <sub>2</sub> -phenyl	4-(NH-BHPA) 5-OH-1-SO <sub>3</sub> H		80%	red	blue	7.10	521 608	540
A <sub>11</sub> -BHPA-SO	2-CN-4,6-d-INO <sub>2</sub> -phenyl	4-(NH-BHPA-SO) 5-OH-1-SO <sub>3</sub> H		57%	red	blue	6.50	518 624	550
A <sub>11</sub> -BHPA	2-F-4,6-d-INO <sub>2</sub> -phenyl	4-(NH-BHPA) 5-OH-1-SO <sub>3</sub> H	26%	92%	red	blue	6.60	520 609	545
A <sub>13</sub> -BHPA	2,6-diCl-4-NO <sub>2</sub> -phenyl	4-(NH-BHPA) 5-OH-1-SO <sub>3</sub> H	44%	65%	red	blue		516 512	
A <sub>16</sub> -BHPA	2,6-diBr-4-NO <sub>2</sub> -phenyl	4-(NH-BHPA) 5-OH-1-SO <sub>3</sub> H	53%	67%	red	red	6.50	518 508	
A <sub>17</sub> -BHPA	6-Br-2-CN-4-NO <sub>2</sub> -phenyl	4-(NH-BHPA) 5-OH-1-SO <sub>3</sub> H	20%	91%	red	blue	7.60	521 624	545
M <sub>1</sub> -BHPA	2-COOH-4,6-d-INO <sub>2</sub> -phenyl	4-(NH-BHPA) 5-OH-1-SO <sub>3</sub> H	2%	48%	red	purple	8.70	519 577	560

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## WE CLAIM:

1. A multilayer sensing element for analyzing the pH and, optionally, pCO<sub>2</sub> of fluids, comprising:

a dye film layer comprising a polyurethane hydrogel in which 0.1-20 weight percent of the polymer units are derived from copolymerization of a reactive azo dye having a pK<sub>a</sub> of from 6 to 8 and exhibiting absorbance of visible light that reversibly shifts as a function of pH,

a reflective film adjacent the dye film comprising a polyurethane hydrogel impregnated with a visible light-reflecting pigment,

a light absorbing film adjacent the light reflecting film comprising a polyurethane hydrogel impregnated with visible light-absorbing pigment, and

optionally, a gas permeable film comprising a silicone polymer.

2. A sensing element according to claim 1 wherein the reactive azo dye is of the general formula:



wherein:

R<sup>2</sup> represents a phenyl, naphthyl, or a C<sub>2</sub>-C<sub>12</sub> heterocyclic aromatic radical, which may be substituted with one or more groups selected from nitro, cyano, sulfo, carboxy, carboxamido, carboalkoxy, acyl, alkoxy, perfluoroalkyl, or halogen atoms such as bromine, chlorine, fluorine, etc.;

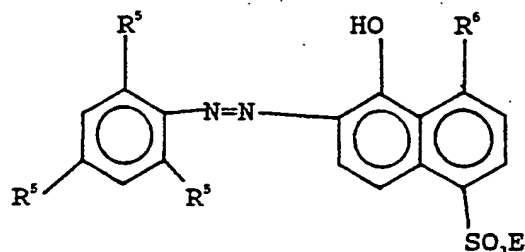
R<sup>3</sup> represents a suphonated naphthol or sulphonated aminonaphthol radical; and

R<sup>4</sup> represents a reactive substituent capable of binding the dye molecule to a polymeric substrate

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without affecting the pH-indicating character of the dye.

3. A sensing element according to claim 2, wherein the azo dye has the formula:



wherein:

Each  $R^5$  is independently selected from hydrogen, halogen, perfluoroalkyl of 1 to 4 carbon atoms, nitro, sulfo, cyano, carboxy, carboalkoxy of 1 to 4 carbon atoms, carboxamido, or acyl, provided that at least one  $R^5$  is nitro or cyano;

$R^6$  is amino, carboxamido, acrylamido,  $-NHCO(CH_2)(CH_2OH)_2$ ,  $-NHCOCH_2NR^7R^8$  or  $-NHCOCH(NR^7R^8)CH_2NR^7R^8$ , wherein  $R^7$  and  $R^8$  are, independently, hydrogen, straight chain or branched alkyl, aminoalkyl, or hydroxyalkyl groups of 1 to 6 carbon atoms, or  $R^7$  and  $R^8$  taken together form the radical  $-CH_2CH_2NR^9CH_2CH_2-$ , wherein  $R^9$  is hydrogen or any of the aforementioned C-C aminoalkyl or hydroxy-alkyl radicals; and E is hydrogen, sodium, lithium, potassium, magnesium or calcium.

4. A sensing element according to claim 3 wherein  $R^5$  para to the azo group and one  $R^5$  ortho to the azo group are nitro or cyano.

5. A sensing element according to claim 4 wherein at least one  $R^5$  ortho to the azo group is a halogen.



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6. A sensing element according to claim 4 wherein  $R^6$  is  $-NHCO(CH_2)(CH_2OH)_n$ ,  $-NHCOCH_2NR^7R^8$  amino, or  $-NHCOCH(CH_2NR^7R^8)NR^7R^8$ .

7. A sensing element according to claim 3 wherein  $R^5$  para to the azo group is nitro, and  $R^6$  is  $-NHCO(CH_2)(CH_2OH)_n$ .

8. A sensing element according to claim 1 wherein the azo dye exhibits a pH-dependent color change with absorption maxima at wavelengths longer than 500 nm for both the protonated and deprotonated forms of the dye.

9. A sensing element according to claim 1 wherein the azo dye is prepared via a coupling reaction between diazonium salts and aminonaphthol sulfonic acids.

10. A sensing element according to claim 9 wherein the diazonium salts are selected from 2-halo-4,6-dinitrophenyl-diazonium salts, 2-halo-6-cyano-4-nitrophenyl-diazonium salts and their substituted analogs.

11. A sensing element according to claim 9 wherein the azo dye is prepared from the coupling reaction of 2-bromo-4,6-dinitrophenyldiazonium sulfate, 2-chloro-4,6-dinitrophenyl-diazonium sulfate, 2-fluoro-4,6-dinitrophenyldiazonium sulfate or 2-trifluoromethyl-4,6-dinitrophenyldiazonium sulfate and amides derived from aminonaphthol sulfonic acids.

12. A sensing element according to claim 11 wherein the sulfonic acids are 6-amino-7-hydroxy-

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2-naphthalenesulfonic acid, 6-amino-4-hydroxy-2-naphthalenesulfonic acid, 4-amino-5-hydroxy-1-naphthalenesulfonic acid, 7-amino-4-hydroxy-2-naphthalenesulfonic acid, 4-amino-5-hydroxy-2,7-naphthalenesulfonic acid or 3-amino-5-hydroxy-2,7-naphthalenesulfonic acid.

13. A sensing element according to claim 3 wherein the azo dye has a pKa value between 6 and 8 and undergoes yellow/orange/red to purple/blue color change with increasing pH.

14. A sensing element according to claim 2 wherein R<sup>2</sup> bears hydroxy, nitro, cyano, sulfo, carboxy, carboxamido, carboalkoxy, acyl, methyl or methoxy substituent groups.

15. A sensing element according to claim 1 wherein said light-reflecting pigment is titanium dioxide.

16. A sensing element according to claim 1 wherein said light-absorbing pigment is carbon black.

17. A multilayer sensing element for analyzing the pH and, optionally, pCO<sub>2</sub> of fluids, comprising:

a dye film layer comprising a polyurethane hydrogel in which 0.1-20 weight percent of the polymer units are derived from copolymerization of a reactive azo dye having a pK<sub>a</sub> of from 6 to 8 and exhibiting absorbance visible light that reversibly shifts as a function of pH,

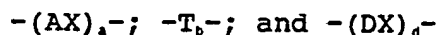
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a reflective film adjacent the dye film comprising a polyurethane hydrogel impregnated with titanium dioxide,

a light absorbing film adjacent the light reflecting film comprising a polyurethane hydrogel impregnated with carbon black, and

optionally, a gas permeable film comprising a silicone polymer.

18. A polyurethane hydrogel dye film comprising a copolymerization product of alkylene glycols or thioglycols, organic polyisocyanates, and hydroxyfunctional or isocyanatefunctional azo dyes, the hydrogels having a three-dimensional network structure including polymeric units of the following general formulas:



wherein:

AX represents a urethane derived by the reaction of a polyalkylene glycol or thioglycol A of molecular weight 200 to 15,000 with an organic diisocyanate or triisocyanate X; T represents a trifunctional or tetrafunctional unit derived from an organic polyol or an organic polyisocyanate; DX represents a unit derived by reacting an azo dye D with an organic poly-isocyanate X and a, b and d are positive integers selected so that the molar ratios of A:X:T are from 2:2:1 to 12:12:1, and so that the D component makes up 0.1% to 20% by weight of the dye film.

19. A dye film according to claim 18, wherein the film is hydrated 40% to 80%.

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20. A dye film according to claim 18, wherein the crosslinking component of the hydrogel is a polysaccharide.

21. A dye film according to claim 18, wherein the polyalkylene glycols are poly(C<sub>2</sub>-C<sub>6</sub>)-alkylene glycols.

22. A dye film according to claim 18, wherein the glycols are polyethylene glycol, polypropylene glycol, polytetramethylene ether glycols, mixtures of such glycols or their corresponding thioglycols.

23. A dye film according to claim 18, wherein the molecular weight of the polyalkylene glycols or thioglycols is from 600 to 3,000.

24. A dye film according to claim 18, wherein the organic polyisocyanates have the general formula:



wherein:

R is an aliphatic hydrocarbon radical of from 4 to 12 carbon atoms, a cycloaliphatic hydrocarbon radical of from 6 to 15 carbon atoms, or an aromatic or aromaliphatic hydrocarbon radical of 6 to 16 carbon atoms, and wherein x represents 2, 3 or 4.

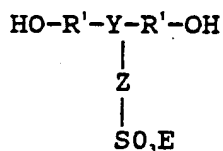
25. A dye film according to claim 24 wherein the organic polyisocyanates are selected from tetramethylene diisocyanate, hexamethylene diisocyanate, 1,4-cyclohexane diisocyanate, 1,4-pentane diisocyanate, methylene bis(4-isocyanatoethyl cyclohexane) 1-isocyanato-3,3,5-trimethyl-5-

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isocyanatomethyl cyclo-hexane, 1,4-isocyanatomethyl cyclohexane, 1,4-isocyanatoethyl cyclohexane, 1,3-isocyanatomethyl cyclohexane, 1,3,5-triisocyanatocyclohexane, diisocyanatopentylcyanomethine, 1,4-diisocyanobenzene, 2,6-diisocyanatotoluene, 2,6-diisocyanatotoluene, toluene diisocyanate (2,4- and 2,6-mixtures), 4,4'-dicyanodiphenylmethane, 4,4'-diisocyanodiphenyl propane (2,2), and p-xylylene diisocyanate.

26. A dye film according to claim 18, which further includes polymeric units of the formula  $-(BX)-$ , derived by reacting an organic polyisocyanate X with an ionic diol or corresponding acid diol B, selected from sulfonate diols, quaternary ammonium diols, phosphate diols, carboxylate diols, or their corresponding carboxylic acids, wherein the polymeric units  $-(BX)-$  make up no more than about 40% by weight of the final hydrogel polymer.

27. A dye film according to claim 26 wherein B represents a sulfonate diols of the formula:



wherein:

R' may be the same or different bivalent aromatic or aliphatic radical of from 1 to 12 carbon atoms;

Y is nitrogen, carbon, sulfur, silicon, or phosphorus, with any free valencies being taken by hydrogen or halogen atoms;

Z is a direct bond or R'; and

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E is hydrogen, sodium, lithium, potassium, magnesium or calcium.

28. A dye film according to claim 27 wherein the diols include N,N-bis(2-hydroxyethyl)-2-N-methylamine ethane sulfonic acid, 1,4-butanediol-2-sulfonic acid, and salts thereof.

29. Azo dyes of the general formula:



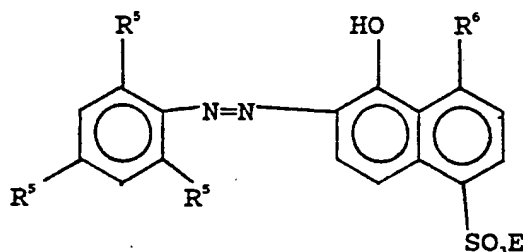
wherein:

$R^2$  represents a phenyl, naphthyl, or a  $C_6$ - $C_{12}$  hetero-cyclic aromatic radical, which may be substituted with one or more groups selected from nitro, cyano, sulfo, carboxy, carboxamido, carboalkoxy, acyl, alkoxy, perfluoroalkyl, or halogen atoms;

$R^3$  represents a sulphonated naphthol or sulphonated aminonaphthol radical; and

$R^4$  represents a reactive substituent capable of binding the dye molecule to a polymeric substrate.

30. Azo dyes of the formula:



wherein:

Each  $R^5$  is independently selected from hydrogen, halogen, perfluoroalkyl of 1 to 4 carbon atoms, nitro, sulfo, cyano, carboxy, carboalkoxy of 1

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to 4 carbon atoms, carboxamido, or acyl, provided that at least one R<sup>5</sup> is nitro or cyano;

R<sup>6</sup> is amino, carboxamido, acrylamido, -NHCO(CH<sub>2</sub>)(CH<sub>2</sub>OH),, or -NHCOCH(NR<sup>7</sup>R<sup>8</sup>)CH<sub>2</sub>NR<sup>7</sup>R<sup>8</sup>, wherein R<sup>7</sup> and R<sup>8</sup> are, independently, hydrogen, straight chain or branched alkyl, aminoalkyl, or hydroxyalkyl groups of 1 to 6 carbon atoms,

or R<sup>7</sup> and R<sup>8</sup> taken together form the radical -CH<sub>2</sub>CH<sub>2</sub>NR<sup>9</sup>CH<sub>2</sub>CH<sub>2</sub>-, wherein R<sup>9</sup> is hydrogen or any of the aforementioned C<sub>1</sub>-C<sub>6</sub> aminoalkyl or hydroxy-alkyl radicals; and

E is hydrogen, sodium, lithium, potassium, magnesium or calcium.

31. An azo dye according to claim 30 wherein R<sup>5</sup> para to the azo group is a nitro group or a cyano group.

32. An azo dye according to claim 31 wherein at least one R<sub>i</sub> ortho to the azo group is a halogen.

33. An azo dye according to claim 31 wherein R<sup>6</sup> is -NHCO(CH<sub>2</sub>)(CH<sub>2</sub>OH),, -NHCOCH<sub>2</sub>NR<sup>7</sup>R<sup>8</sup> amino, or -NHCOCH(CH<sub>2</sub>NR<sup>7</sup>R<sup>8</sup>)NR<sup>7</sup>R<sup>8</sup>.

34. An azo dye according to claim 30 wherein R<sup>5</sup> para to the azo group is nitro, and R<sup>6</sup> is -NHCOCH(CH<sub>2</sub>NR<sup>7</sup>R<sup>8</sup>)NR<sup>7</sup>R<sup>8</sup>, and R<sup>7</sup> and R<sup>8</sup> are aminoethyl, aminopropyl, hydroxyethyl, hydroxypropyl, or tris (hydroxymethyl)methyl.

35. An azo dye according to claim 30 wherein R<sup>5</sup> para to the azo group is a nitro or cyano group and R<sup>6</sup> is -NHCO(CH<sub>2</sub>)(CH<sub>2</sub>OH),.

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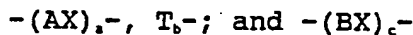
36. An azo dye according to claim 30 wherein the starting materials include 2-halo-4,6-dinitrophenyldiazonium salts, 2-halo-6-cyano-4-nitrophenyldiazonium salts and their substituted analogs.

37. An azo dye according to claim 36 prepared from the coupling reaction of 2-bromo-4,6-dinitrophenyldiazonium sulfate, 2-chloro-4,6-dinitrophenyldiazonium sulfate, 2-fluoro-4,6-dinitrophenyldiazonium sulfate, 2-trifluoromethyl-4,6-dinitrophenyldiazonium sulfate and amides derived from aminonaphthol sulfonic acids.

38. An azo dye according to claim 37 wherein the sulfonic acids are 6-amino-7-hydroxy-2-naphthalene-sulfonic acid, 6-amino-4-hydroxy-2-naphthalenesulfonic acid, 4-amino-5-hydroxy-1-naphthalenesulfonic acid, 7-amino-4-hydroxy-2-naphthalenesulfonic acid, 4-amino-5-hydroxy-2,7-naphthalenesulfonic acid and 3-amino-5-hydroxy-2,7-naphthalenesulfonic acid.

39. An azo dye according to claim 29 wherein R<sup>2</sup> bears hydroxy, nitro, cyano, sulfo, carboxy, carboxamido, carboalkoxy, acyl, methyl or methoxy substituent groups.

40. Hydrogels comprising a three-dimensional network structure including polymeric units of the following general formulas:



wherein:

AX represents a urethane derived by the reaction of a polyalkylene glycol or thioglycol A of



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molecular weight 200 to 15,000 with an organic diisocyanate or triisocyanate X;

T represents a trifunctional or tetrafunctional unit derived from an organic polyol or an organic polyisocyanate;

BX represents a unit derived by reacting an organic polyisocyanate X with a sulfonate diol, phosphate diol, carboxylate diol, or quaternary ammonium diol B; and

a, b, and c are integers, a and b being greater than zero, and a, b, and c being selected so that the  $-(BX)-$  units make up no more than 40% by weight of the hydrogel and the molar ratios of A:X:T in the final polymer are from 2:2:1 to 12:12:1.

41. Hydrogels according to claim 40 which may be hydrated 20% to 90%, with uniform water distribution and elastomeric properties.

42. Hydrogels according to claim 40, wherein the polyalkylene glycols and thioglycols for use in preparing the hydrogels are poly( $C_2-C_6$ )alkylene glycols and thioglycols selected from polyethylene glycol, polypropylene glycol, polytetramethylene ether glycols, mixtures of such glycols and their corresponding thioglycols.

43. Hydrogels according to claim 42, wherein the molecular weight of the polyalkylene glycols or thioglycols is from 200 to 15,000.

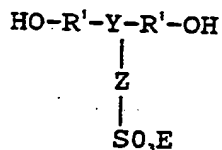
44. Hydrogels according to claim 40, wherein the organic polyisocyanates have the general formula  $R(N=C=O)_x$ , wherein R is an aliphatic hydrocarbon radical of from 4 to 12 carbon atoms, a cycloaliphatic

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hydrocarbon radical of from 6 to 15 carbon atoms, or an aromatic or aromaliphatic hydrocarbon radical of 6 to 16 carbon atoms, and wherein x represents 2, 3 or 4.

45. Hydrogels according to claim 44, wherein the polyisocyanates are selected from tetramethylene diisocyanate, hexamethylene diisocyanate, 1,4-cyclohexane diisocyanate, 1,4-pentane diisocyanate, methylene bis(4-isocyanatoethyl cyclohexane), 1-isocyanato-3,3,5-trimethyl-5-isocyanatomethyl cyclohexane, 1,4-isocyanatomethyl cyclohexane, 1,4-isocyanato-ethyl cyclohexane, 1,3-isocyanatomethyl cyclohexane, 1,3,5-triisocyanatocyclohexane, diisocyanatopentylcyanomethine, 1,4-diisocyanobenzene, 2,6-diisocyanatotoluene, 2,6-diisocyanatotoluene, toluene diisocyanate (2,4- and 2,6-mixtures), 4,4'-dicyanodiphenylmethane, 4,4'-diisocyanodiphenyl propane (2,2), and p-xylylene diisocyanate.

46. Hydrogels according to claim 40 wherein the sulfonate diols include compounds of the formula:



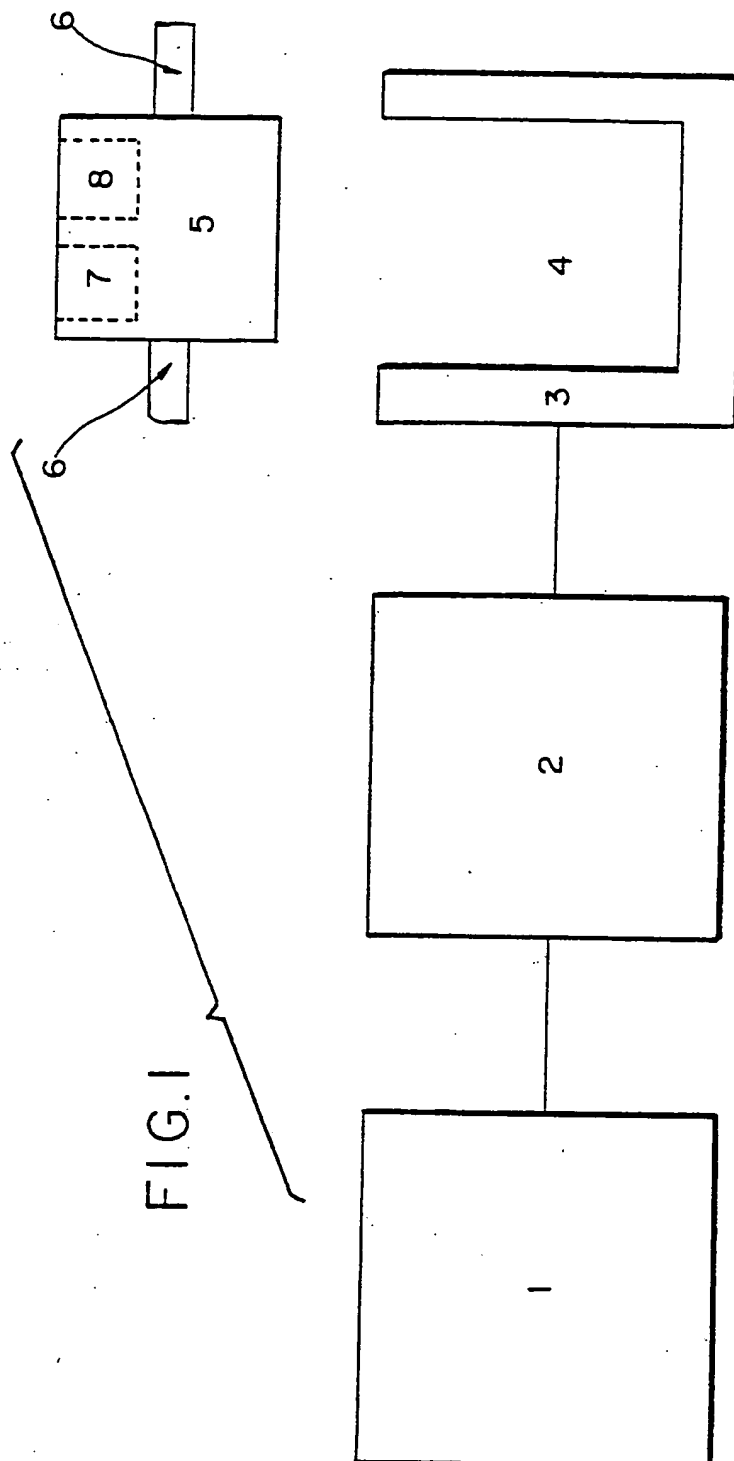
wherein:

R' may be the same or different bivalent aromatic or aliphatic radical of from 1 to 12 carbon atoms;

Y is nitrogen, carbon, sulfur, silicon, or phosphorus, with any free valencies being taken by hydrogen or halogen atoms;

Z is a direct bond or R'; and

E is hydrogen, sodium, lithium, potassium, magnesium or calcium.



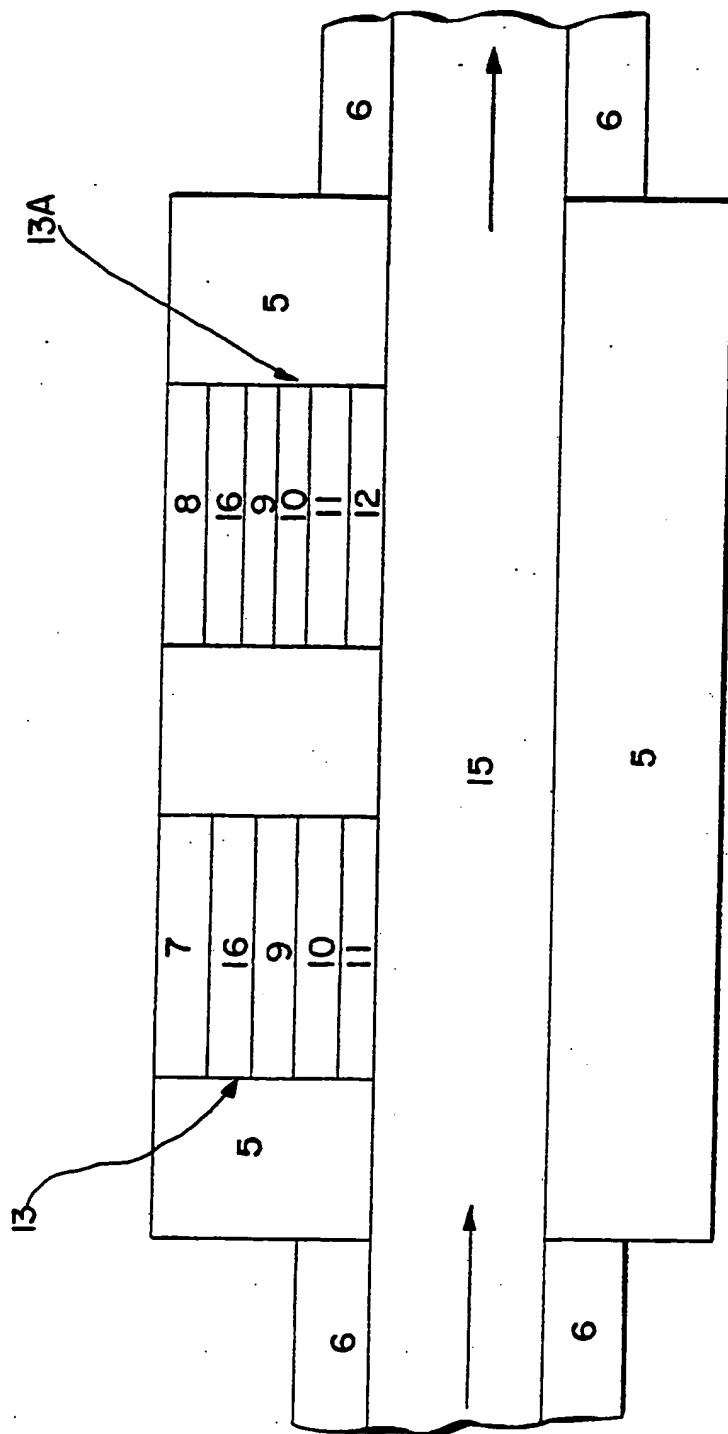


FIG. 2

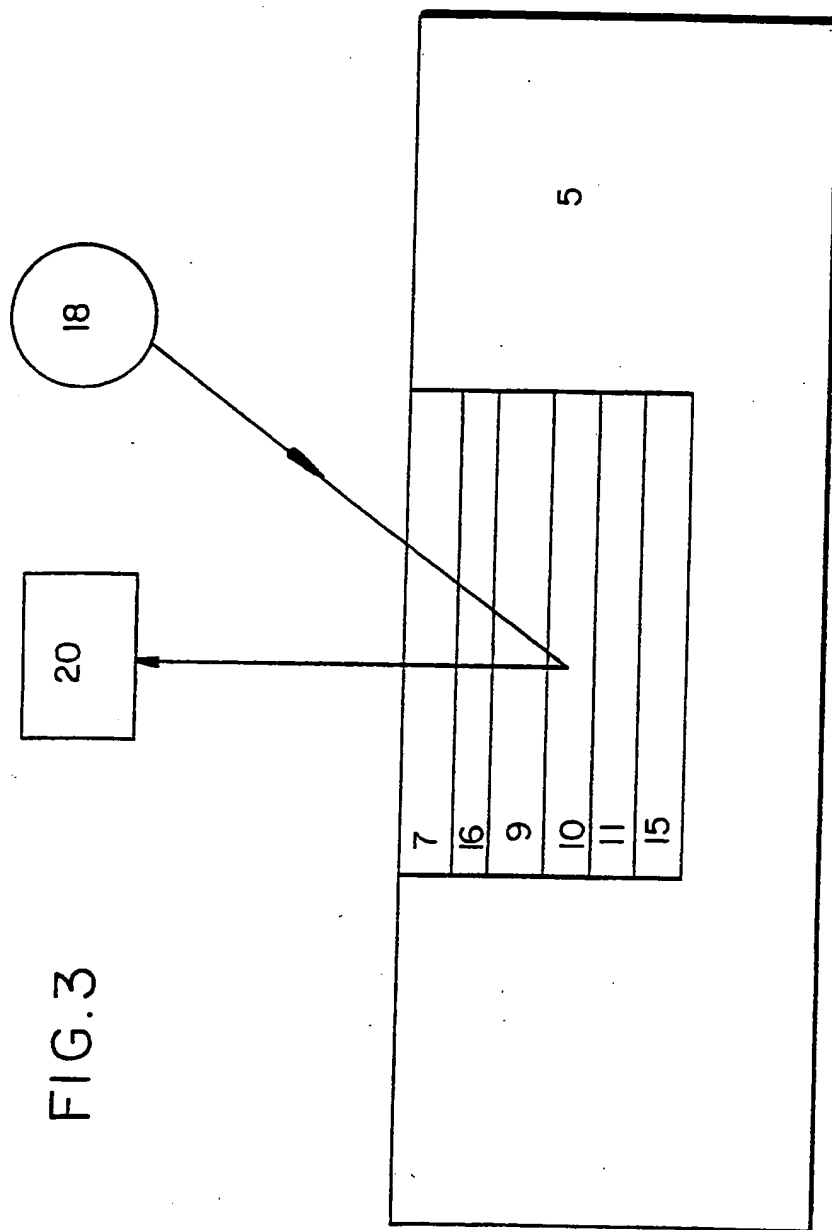
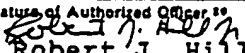


FIG. 3

# INTERNATIONAL SEARCH REPORT

International Application No. **PCT/US89/03015**

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (If several classification symbols apply, indicate all) <sup>1</sup>		
According to International Patent Classification (IPC) or to both National Classification and IPC <b>IPC(4): C08G 18/32, 18/38; G01N 21/78, 33/50</b> <b>U.S. CL.: 422/56; 436/68, 133, 163, 170; 528/71, 85; 534/643, 862, 878</b>		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>4</sup>		
Classification System	Classification Symbols	
U.S.	422/55-60; 435/805; 436/68, 133, 163, 169, 170; 528/71, 85; 534/643, 862, 878	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>4</sup>		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT <sup>11</sup></b>		
Category <sup>8</sup>	Citation of Document, <sup>16</sup> with indication, where appropriate, of the relevant passages <sup>17</sup>	Relevant to Claim No. <sup>18</sup>
A	US, A, 3,142,669 (FEEMAN) 28 July 1964, see the entire document.	
A	US, A, 3,420,635 (DAVIS) 07 January 1969, see the entire document.	
A	US, A, 3,544,546 (CRABTREE et al) 01 December 1970, see the entire document.	
A	US, A, 3,928,292 (MAEDA et al) 23 December 1975, see the entire document.	
<div style="display: flex; justify-content: space-between;"> <div style="width: 48%;"> <p><sup>12</sup> Special categories of cited documents: <sup>13</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 48%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p> </div> </div>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search <sup>1</sup>		Date of Mailing of this International Search Report <sup>1</sup>
09 September 1989		11 OCT 1989
International Searching Authority <sup>1</sup>		Signature of Authorized Officer <sup>19</sup>
ISA/US		 Robert J. Hill, Jr.

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